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HENRY HORATIO DIXON

(WITH ONE PLATE)

HENRY HORATIO DIXON was born in Dublin on May 19, 1869, the youngest of seven brothers. The family included also two sisters. The father, GEORGE DIXON, owned a soap works, which is still carried on successfully; one of his brothers was a Fellow of Trinity College. Seldom do seven brothers have such distinguished careers, six in their native city, for in Ireland most families have some members abroad. Two of them carried on their father's enterprise, one was a barrister, one chief inspector of technical schools, and three were university professors, in engineering, anatomy, and botany. Mr. DIXON died while his children were still young, so the burden of upbringing fell on his widow, REBECCA, daughter of GEORGE YEATES of Dublin. She made a home for all and had a welcome for their friends, and later for their pupils. When one considers this large family, so hospitable, so full of interest in many subjects, it becomes clear that in the quiet old lady with her smile of welcome and her lively mind lay the secret of it all.

After attending Rathmines School, HENRY DIXON entered Trinity College, the sole college (founded by Queen Elizabeth in 1591) of the University of Dublin. He obtained a classical scholarship and took prizes in Italian. In 1892 he graduated with first class honours in natural science, which in Dublin meant botany, zoology and geology. He later studied at Bonn under the celebrated STRASBURGER. In 1894 he was appointed assistant to Prof. E. P. WRIGHT and he succeeded to the University Chair of Botany when WRIGHT retired in 1904. WRIGHT had travelled extensively, and his department contained a fine herbarium, with many of his predecessor W. H. HARVEY'S type specimens of algae. There was also a beautiful and well stocked botanic garden, so ample opportunity existed for morphological and cytological research. In 1906 DIXON became director of the botanic gardens, and after WRIGHT'S death in 1910, of the herbarium also.

No account of DIXON'S moulding would be understandable without mention of his friend JOHN JOLY, his senior by some fourteen years. The fam-

ilies were neighbours, and later both moved to adjacent suburban homes. JOLY, the brilliant physicist, found his young neighbour a congenial companion on his yachting cruises. The benefit to a botanist of being able to discuss physiological problems with a competent physicist, was of course great. Thus the names JOLY and DIXON were linked for ever as the originators of the cohesion theory of the ascent of sap. In many university and other activities their names were also associated, and an ideal friendship of fifty years was terminated in 1934 by JOLY'S death. To his friend he left his house, his pictures, his books, and it was upon DIXON that the Royal Society of London called to write an outline of JOLY'S life.

In 1907 Prof. DIXON married DOROTHEA MARY, daughter of the late Sir JOHN H. FRANKS, C.B., and they have three sons. The eldest, G. JOLY DIXON, is a Member of the Royal College of Physicians at the Charing Cross Hospital, London; the second, Dr. KENDAL C. DIXON, is a Fellow of Kings College, Cambridge; the youngest is still at school. In their own home Prof. and Mrs. DIXON have continued the hospitable tradition of the family.

Outside the university, DIXON'S activities have been largely connected with the Royal Dublin Society, which fosters scientific research, agriculture, music, distributes radium emanation, and runs the famous Dublin Horse Show. Of this Society he is a vice-president. He is also on the Committee of the Imperial Bureau of Mycology, London, and is a Commissioner of Irish Lights. The latter involves a yearly inspection of light houses and lightships, a service still maintained by the British Government. To be hauled up a light house and lowered again into a small boat in the grip of Atlantic rollers is no nominal undertaking.

In 1908 DIXON was elected to the Fellowship of the Royal Society of London, and in 1917 he received the Boyle Medal of the Royal Dublin Society. In 1927 he went to the U. S. A. as visiting professor of the University of California. The beauty of the country, the vast scale of the university, the keen scientific discussions, and the warmth of the personal welcome made a deep impression upon him. In this tour he was accompanied by Mrs. DIXON and it was marked in their family history by the christening of their youngest son as HENRY BERKELEY, born in 1928.

In 1932 DIXON was elected a Corresponding Member of the Botanical Society of America, an honour which brought him pleasant memories of his American friends.

When he reached seventy, this year, DIXON'S Irish pupils decided that his birthday, May 19th, should be signalized by a congratulatory address. The suggestion received a ready response and, including some foreign lists which unavoidably came in late, an address with about 600 signatures was presented to him by the Provost of Trinity College at an informal gathering. This testimony to his work, from so many friends and fellow workers in so

many lands, gave him very genuine pleasure. The members of the University also were most appreciative of the honour paid to their professor from abroad.

A list of DIXON's publications may be found in the account of the award of the Boyle Medal, in the Scientific Proceedings of the Royal Dublin Society, 1917, vol. **15**, pp. 179–184, obtainable separately as publication no. 19 of the volume. This list of 61 titles includes *Transpiration and the Ascent of Sap in Plants,* Macmillan, London, 1914. In 1924 DIXON delivered three lectures on *The Transpiration Stream* before the University of London. These were published by the University Press. Recently his Royal Society Croonian Lecture entitled *Transport of Substances in Plants* reviewed the subject in all its aspects. It is reprinted in Notes from the Botanical School of Trinity College, Dublin, vol. 4, no. 6, November, 1938. Most of DIXON's papers, which have chiefly appeared in Sci. Proc. Roy. Dublin Soc., have been reprinted in the Notes, which was started in 1896, by Prof. E. P. WRIGHT. Since the Boyle Medal award list, twenty-one additional papers have been re-printed in the Notes.

When DIXON was appointed to the Chair of Botany the department consisted of a few rooms in a residential house in the Front Square, with the herbarium in large rooms on the top. Lectures to the classes of medical students and practical classes were held elsewhere. Through the generosity of Lord Iveagh and a number of graduates of the University of Dublin a new School of Botany was erected in 1907. It bears throughout marks of the forethought of the Professor. No building ever had less space wasted in passages, etc., or rendered useless by inadequate lighting.

The elementary lectures are always delivered by the Professor; each is followed by a practical class in which his assistants and demonstrators teach under his supervision. The class also visits the Gardens. This course was published in 1922 as *Practical Plant Biology* (Longmans, Green & Co.). Starting with the use of the microscope, it deals first with the structure and physiology of unicellular plants and bacteria, and simple cultures are made. The medical student is thus, at the start of his career, brought to understand the meaning of infection and sterilization. Special attention is paid to the real understanding of the sizes of objects seen under the microscope, measurements being made in three dimensions. Types of algae and fungi are then considered, followed by the liverworts, ferns, gymnosperms, and angiosperms. The whole course is constructed to illustrate evolution, and the final lectures deal with this, nuclear division, and theories of heredity. Thus one does not wander from the green leaf, root, and flower back to the unicellular organisms, but progresses mentally in the same direction as was followed in plant evolution and is followed in its life history by every plant. This course is recognised by past students, especially those of the medical

profession, as constituting a landmark in their education; and its creation must be reckoned as DIXON's greatest success as a teacher. Those taking natural science for a degree specialize, in the latter half of the four years course, in one subject. The very limited numbers insure that each student receives that personal contact which can prove so stimulating; and old students who return have the pleasure of realizing that the courses are undergoing a ceaseless revision, and are being illustrated by new lantern slides or by the professor's new lecture experiments or beautiful cytological preparations.

Though not a systematist, DIXON is fully aware of the importance of this branch of botany. Consequently, soon after WRIGHT's death he succeeded in having an adequate addition made to the east end of the School; so that by 1912 the Herbarium was housed better than ever before, in a well lighted room, together with many floristic works. The labour of moving, sorting, and treating hundreds of bundles to destroy insects was immense, and was carried out with but little assistance.

The duties of Director of the Botanic Gardens have not been interpreted by DIXON as a sinecure. The beautiful gardens, about a mile from the College, were walled in early in 1807, and in the spring of 1808 the planting of trees, shrubs, and herbaceous plants commenced. They were thus old and well stocked when DIXON took charge, yet he has improved them and added over seven thousand species. One notable addition may be mentioned, a collection of Siamese orchids made over a number of years by A. F. G. KERR, Esq., M.D.

One thinks of DIXON as a physiologist, but in his earlier years he made important contributions to cytology; these include work on the chromosomes and the first mitosis of the spore-mother-cells of Lilium and on the nuclei of the endosperm of *Fritillaria imperialis*. He also studied the resistance of seeds to high and low temperatures and to poisons, including radium emanation. His early experience when studying the temperature of the underground organs of plants led to his thermoelectric method of cryoscopy, used in so much of his later work on osmotic pressure. As a memory of his love of sailing we have his Royal Society paper on the structure of Coccospheres and the origin of Coccoliths (1900).

During the war DIXON undertook the examination of over forty varieties of wood known commercially as mahogany. This paper, with 138 plate figures, was of technical interest since mahogany was used for aeroplane propellers.

In 1922 DIXON was President of the Botanical Section of the British Association; his address dealt with the vexed question of the transport of organic substances in plants, a problem soon to be greatly clarified by the work of his pupil T. G. MASON. With his assistant N. G. BALL, DIXON

then published an account of the channels of transport from the storage organs of seedlings of Lodoicea, Phoenix, and Vicia. No account of the subject should omit consideration of this paper, in which transport of carbohydrates is considered in an organ where the transport of water is unnecessary.

DIXON'S work on variation in the permeability of leaf-cells appeared in 1924. Later, with T. A. BENNET-CLARK, he studied the responses of plant tissues to electric currents and the influence of temperature. This led to an investigation of the electrical properties of oil-water emulsions in relation to the structure of the plasmatic membrane. By 1932 highly important conclusions had resulted from this investigation.

In 1933 DIXON published a paper on bast sap, in which the theory of MÜNCH was specially considered, and he subsequently made an extensive study of the convection of heat and materials in the stem of a tree. In 1938 a paper on subaqueous transpiration appeared, and his 70th birthday found DIXON still busy with his researches, and with his junior class of one hundred.

RESPIRATION OF ACORNS AS RELATED TO TEMPERATURE AND AFTER-RIPENING[1]

JAMES W. BROWN

(WITH TEN FIGURES)

Introduction

It is well known that while acorns of the white oak group will germinate as soon as they fall from the tree, acorns of the red oak group will not germinate until they have undergone a period of after-ripening. Although the red oak acorns are morphologically mature they must undergo certain physiological changes before they will germinate even under the most favorable conditions. The nature of the physiological changes occurring during the after-ripening of acorns is not fully understood. KORSTIAN (**6**) found that after-ripening is affected by temperature. Storage at an average night temperature of 50° F. (10.0° C.) and day temperature of 65° F. (18.3° C.) resulted in the most satisfactory after-ripening as judged by promptness of germination and the percentage finally germinating.

It appears that certain chemical and physiological changes occur in red oak acorns during over-wintering and the accompanying after-ripening which do not occur in acorns of the white oak group. It was believed that concurrent measurements of the O_2 consumption and CO_2 production of acorns of the white and red oak groups might supply some information concerning differences in the physiology of the two groups. It also seemed possible that periodic measurements of the rate of respiration and the respiratory quotient of red oak acorns stored at various temperatures would indicate the effects of storage temperature on the rate of after-ripening and the accompanying physiological changes.

Acorns of northern red oak [*Quercus borealis* var. *maxima* (Marsh.) Ashe] and white oak (*Quercus alba* L.) were chosen for these studies because they are widely distributed and representative species of the two principal groups of oaks.

Methods

TREATMENT OF MATERIAL PREPARATORY TO MEASUREMENTS

About 2750 white oak acorns and 2500 northern red oak acorns were collected at the time they fell from the trees in the autumn of 1936 and an equal number was collected in the autumn of 1937. When the last collection was made in late October of 1936, 250 acorns of each species were planted in a greenhouse in a mixture of equal parts of sand and peat. The

[1] This investigation was supported by a Fellowship in Forestry in the Graduate School of Arts and Sciences of Duke University.

remainder of the acorns of both species was placed in the same mixture in flats. The northern red oak acorns were divided into groups and stored at 0°, 10°, and 20° C. The white oak acorns were placed in a cold room at approximately 0° C. These temperatures varied 2° to 3° C. except the storage temperature of 10° which varied only 0.5° C. After six weeks of storage at 0° C., repairs to the cold room necessitated removal of the material to a refrigerator at 2° which varied about 0.5° C.

In 1937, 100 northern red oak acorns and 200 white oak acorns were planted in a mixture of sand and peat in a greenhouse on the day of collection. The white oak acorns to be used in other studies were placed in the sand-peat mixture in flats (KORSTIAN, 7) and stored at 2.5° C. The northern red oak acorns were divided into four groups for storage in the same manner at 2.5°, 12.5°, 15.0°, and 17.5° C. Refrigerators were set at 10° and 15° C. and electric ovens were placed in them to obtain the 12.5° and 17.5° C. temperatures.

In 1936 from 30 to 36 samples were used in a series of determinations, in 1937 only 10 to 18 samples. Each sample consisted of two or three acorns, the number depending on the size. Statistical studies of the 1936 data showed that the smaller number of samples could be used without any appreciable loss in significance of the results. Enough sound acorns to supply the required samples were removed from storage the night before a series of determinations were to be made. The pericarp and any loose part of the testa were removed, the seeds were divided into groups constituting three or four samples, depending upon the conditions of the experiment, then placed in cheesecloth bags, submerged in water, drained a moment and placed in two-quart glass jars. The jars had inner supports of glass and wire about two inches above the level of 150 ml. of 2N NaOH. After the seeds had been placed in the jars, the tops were screwed on and the seeds were kept at the temperature of the experiment until the next day in an atmosphere free of CO_2. When runs were about to be made, the material was removed from storage, one jar at a time, and transferred from the jar to containers in the bath at the same temperature. These containers also had a supply of 2N NaOH to preserve a CO_2-free atmosphere. WILLAMAN and BEAUMONT (**12**) working with apple twigs and wheat in closed containers found that material previously exposed to a high concentration of CO_2 showed an abnormally high CO_2 production when the CO_2 content of the surrounding atmosphere was decreased. This was explained as possibly resulting from CO_2 accumulated in the tissues diffusing out until equilibrium was attained with the surrounding atmosphere. Since, in this study, the determinations of CO_2 production were made in a CO_2-free atmosphere, storage in such an atmosphere resulted in the acorns already being in equilibrium when the respiration determinations were started. At the appropriate time,

each sample was transferred to a respirometer vessel and measurements of respiration were made. The samples were removed and their volumes determined to the nearest 0.1 ml. by direct displacement in water in a specially constructed tube (RUBINSTEIN, 10).

After the volumes of the samples had been recorded, they were placed in weighing bottles which had been brought to constant weight, set in a drying oven, and kept at approximately 103° C. for 24 hours. The samples were then cooled in a desiccator and weighed. The data were then converted into cubic millimeters of gas per gram of dry weight of the acorns.

In those experiments where respiration was measured at a temperature differing from the storage temperature, the material was kept at the experimental temperature for a period of at least 12 hours before making measurements. Preliminary runs made in the spring of 1936 indicated that it took acorns about 6 hours to approach equilibrium after undergoing a change in temperature (fig. 1).

The acorns of both species of oak were ground immediately upon removal from storage for one set of catalase measurements (fig. 8A and table VII) while the acorns were allowed a 24-hour germinating period at 25° C. for another set of measurements (fig. 8B). All catalase measurements were made at 25° C.

RESPIRATION

Warburg respirometers were used to measure the concurrent O_2 absorption and CO_2 production of the acorns in this investigation. The respirometers were immersed in a water bath similar to that described by STIER and CROZIER (11). The bath had a temperature range of from −5° to 35° C. and the total variation at a set temperature was 0.054° C. as measured by a Beckman thermometer.

The principle of the Warburg method is based upon the manometric measurement of changes in gas pressure in a closed system adjustable to constant volume. By placing an alkali in the bottom of the respirometer vessel, it is possible to absorb the CO_2 produced in respiration so that any pressure change shown by the manometer is caused by the consumption of O_2. After a measurement of O_2 absorption has been made, a reading of the CO_2 produced can be obtained by introducing an excess of acid into the alkali from a side arm of the vessel, thus liberating the CO_2 previously absorbed. The details of this method as applied to plant material have been described by BROWN (4).

CATALASE

The technique and apparatus described by LOOMIS and SHULL (8) were employed with certain modifications in studying the catalase activity of the two species of acorns. Two or three acorns were ground with quartz sand

for three minutes and made up to a 10 per cent. suspension by the addition of distilled water. From this suspension 5 samples of 4 ml. each were drawn. A 4-ml. sample was placed in one arm of a catalase tube while 4 ml. of H_2O_2 were placed in the other arm. The tube was attached to the apparatus, brought to the temperature of the bath (25° C.), the manometer levelled and read. A check or control measurement for any change in concentration of the H_2O_2 during the progress of the experiment was made by determining the volume of O_2 produced by 2 ml. of H_2O_2 and 0.5 gm. of MnO_2. The mean of 4 consecutive measurements was obtained and adjusted to 1 gm. of MnO_2. The means obtained were all approximately 50 ml. of O_2 so each was adjusted to 50 ml. The mean of 5 catalase measurements on the acorn suspension was determined and adjusted for variation in concentration of H_2O_2 by the factor obtained from the control determination.

Experiments and results

Time required to adjust acorns to a new temperature

An investigation was made in the spring of 1936 to determine how long acorns used in respiration studies must be kept at a new temperature in order to insure a constant rate of respiratory activity. Northern red oak acorns were taken from storage at 10° C. and their respiration measured at two-hour intervals for a 10-hour period at 20° C., using the original samples for all measurements. Figure 1 shows that a constant rate of respiration

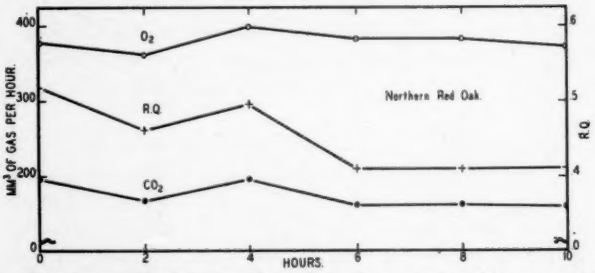

Fig. 1. The time required for northern red oak acorns to attain a constant rate of respiration at a new temperature.

was reached by these acorns in 6 hours. Each point on the graph was determined by the means of 5 samples, except the points at 10 hours, for which only 4 samples were available. It is probable that some physiological adjustment always occurs when material is moved from one temperature to another, and time should be allowed for the attainment of a new equilibrium before making any determinations of respiration at the new temperature. It is also probable that a much longer adjustment period would be needed if the pericarps were not removed from the acorns.

Comparison of respiration of the acorns of two species of oak

Northern red oak and white oak acorns were stored at 0° C. at the time of fall from trees in 1936, and measurements were made of simultaneous O_2 consumption and CO_2 production at intervals over a period of a month during the after-ripening period of the northern red oak acorns. When the acorns fell from the trees in 1937, a similar experiment was started using a storage temperature at 2.5° C. but was continued for two and one-half months.

As shown in figure 2, the respiratory quotients of the two species are very similar indicating that the metabolism of the two is similar. The rate of O_2 consumption of the white oak acorns was from 1.3 to 1.7 times greater

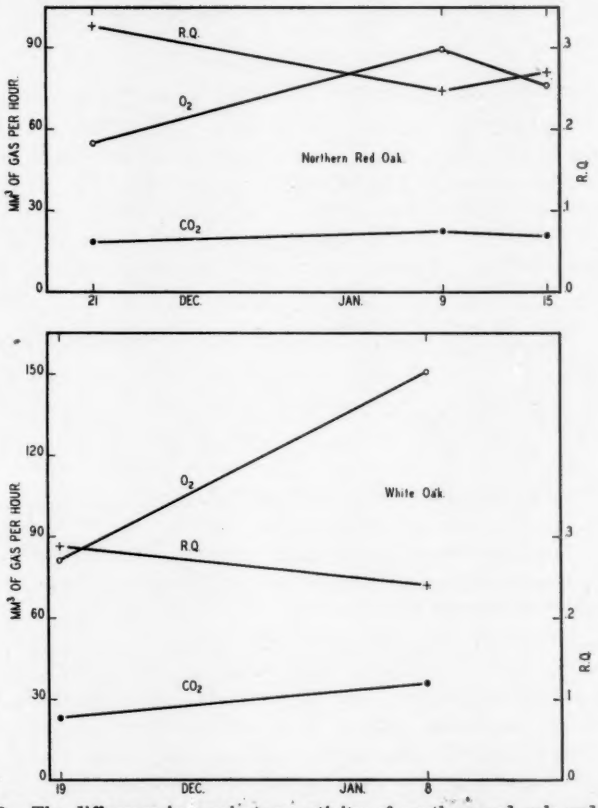

Fig. 2. The difference in respiratory activity of northern red oak and white oak acorns stored and measured at 0° C. in 1936–1937.

than that of the northern red oak acorns. The O_2 consumption of both species increased with length of time in storage while the CO_2 production remained almost constant. From the 9th to the 15th of January the rate of respiration of the northern red oak acorns decreased slightly while the R. Q. remained relatively constant (table I). Measurements of white oak acorns

TABLE I

DIFFERENCE IN THE RESPIRATORY ACTIVITY OF NORTHERN RED OAK AND WHITE OAK ACORNS STORED AND MEASURED AT 0° C.

DATE	GAS	NUMBER OF OBSERVATIONS	MEAN VOLUME OF GAS PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT $\left(\text{R. Q.} = \frac{\text{mean } CO_2}{\text{mean } O_2}\right)$
Northern red oak						
			*mm.*³	*mm.*³	%	
12–21–36	CO_2	34	18.09	1.20	6.66	0.3269
	O_2	34	55.34	4.90	8.85	
1– 9–37	CO_2	36	22.14	1.57	7.09	0.2472
	O_2	36	89.56	6.03	6.73	
1–15–37	CO_2	31	20.50	1.69	8.23	0.2693
	O_2	29	76.12	4.38	5.75	
White oak						
12–19–36	CO_2	36	23.38	1.63	6.96	0.2878
	O_2	36	81.24	6.72	8.27	
1– 8–37	CO_2	35	36.20	2.70	7.46	0.2402
	O_2	35	150.72	5.21	6.91	

were discontinued after the 8th of January because their radicles were so long that it was impossible to place them in respirometer vessels without injury.

Referring to figure 3 and table II, the CO_2 production of white oak acorns was constant during the period of storage at 2.5° C., while the CO_2 production of the northern red oak acorns varied slightly. The O_2 consumption of the northern red oak acorns increased rather sharply from the start of the experiment while the white oak acorns showed a constant rate of O_2 consumption during the first three weeks of storage, a sharp rise during the next three weeks, and a slight decrease in the last three weeks. The R. Q. of the white oak acorns varied inversely as the O_2 consumption since the CO_2 production was constant. The R. Q. of the northern red oak acorns approached very closely that of the white oak acorns during the last 6 weeks of the experiment. There was a difference of about 0.1 in the R. Q.'s of both species during this period.

TABLE II

RESPIRATION OF NORTHERN RED OAK AND WHITE OAK ACORNS STORED AND MEASURED AT 2.5° C.

DATE	GAS	NUMBER OF OBSERVATIONS	GAS PER GRAM DRY WEIGHT PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
				Northern red oak		
			*mm.*³	*mm.*³	%	
11–21–37	CO_2	18	29.94	3.62	12.09	0.4990
	O_2	18	60.00	5.20	8.67	
12–16–37	CO_2	10	9.86	1.94	19.68	0.0613
	O_2	10	160.88	11.98	7.45	
1– 7–38	CO_2	10	17.58	3.30	18.77	0.0871
	O_2	10	201.74	14.22	7.05	
				White oak		
10–31–37	CO_2	18	26.30	2.60	9.89	0.4125
	O_2	18	63.76	1.32	2.07	
11–21–37	CO_2	18	30.74	3.46	11.26	0.4910
	O_2	18	62.60	6.00	9.58	
12–16–37	CO_2	10	28.42	4.06	14.29	0.1636
	O_2	10	173.68	9.72	5.60	
1– 7–38	CO_2	10	25.32	1.46	5.77	0.1594
	O_2	10	158.82	9.04	5.69	

EFFECTS OF TEMPERATURE ON RESPIRATION OF ACORNS

The effect of temperature on the rate of respiration of northern red oak acorns stored at 0° and 10° C. in 1936–1937 was found by measuring their respiration at 0°, 5°, 10°, 15°, 20°, 24° and 30° C. The experiment was repeated in 1937–1938 using both northern red oak acorns and white oak acorns which had been stored at 2.5° C. The respiration of both species was measured at 2.5°, 5°, 10°, 15°, 20°, 25° and 30° C.

Figure 4A shows that the CO_2 production of northern red oak acorns increased along a practically straight line from 5° to 30° C. The O_2 consumption was much less regular in its behavior but generally increased with temperature. The lowest respiratory activity was found at 5° C. The R. Q. of this species increased gradually at the start and more rapidly as the temperature increased from 0° to 24° C., where it reached its maximum of 0.6208 (table III). From this maximum the R. Q. fell to 0.5442 at 30° C.

It should be noted that, in this experiment, the storage temperature for the material measured at 0° and 5° was 0° C. while the material measured from 10° to 30° was stored at 10° C. Lack of material prevented making measurements over the entire temperature range of acorns stored at one temperature.

The respiratory behavior of northern red oak acorns stored at 2.5° and measured over a temperature range from 2.5° to 30° C. is somewhat difficult to explain. The CO_2 production (fig. 5) was essentially the same as in the previous year (fig. 4A) but did not proceed at as great a rate. The O_2 consumption generally decreased with increased temperatures where previously it had generally increased. The R. Q., however, although irregular at first,

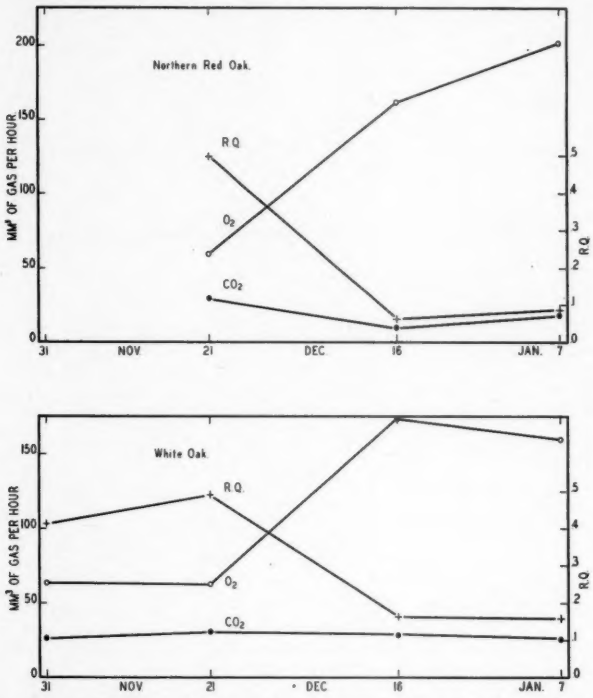

FIG. 3. The respiratory activity of northern red oak and white oak acorns stored and measured at 2.5° C. in 1937–1938.

finally increased regularly with temperature at the higher temperatures (table IV). The R. Q. (fig. 5) of the northern red oak acorns was less than the R. Q. of the preceding year by about 0.2. The material was measured after about the same length of storage both years.

The R. Q. and CO_2 production of the white oak acorns in 1937–1939 (fig. 5) increased regularly while the O_2 consumption varied until it apparently reached an equilibrium at 15° which was held until 25°, and then rose at 30° C. The rate of respiration was generally higher in the white oak acorns than it was in the northern red oak acorns.

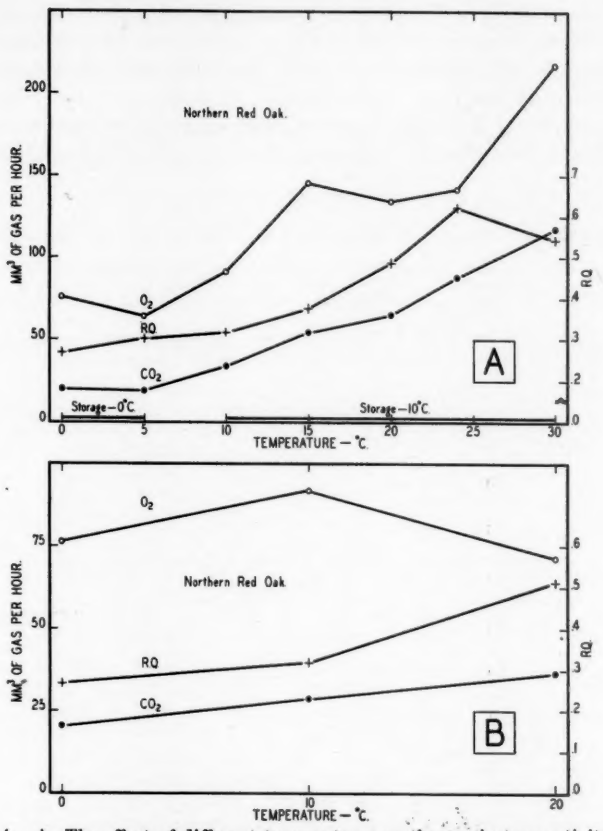

FIG. 4. A. The effect of different temperatures on the respiratory activity of northern red oak acorns after storage at 0° and 10° C. in 1936–1937.

B. The effect of storage temperature on the respiratory activity of northern red oak acorns in 1936–1937. Measurements were made at the storage temperatures.

EFFECT OF STORAGE TEMPERATURES ON R. Q. AND RATE OF RESPIRATION OF NORTHERN RED OAK ACORNS

The effect of storage temperatures on the R. Q. and rate of respiration of northern red oak acorns was investigated by measuring the O_2 consumption and CO_2 production of the acorns at the temperature at which they were stored (0°, 10°, and 20° C. in 1936–1937; 2.5°, 12.5°, 15.0° and 17.5° C. in 1937–1938).

Figure 4B shows a straight line relation between the rates of CO_2 production at 0°, 10°, and 20° C., increasing from the lowest storage tempera-

ture to the highest. The R. Q. increased gradually with temperature in the first stage, and more rapidly in the second stage. The O_2 consumption was practically the same at 0° and 20° but it reached its highest rate at 10° C. storage (table V).

The behavior of the northern red oak acorns the following year was somewhat different insofar as the O_2 consumption and CO_2 production were concerned but the R. Q.'s obtained at the different temperatures of the second year generally resemble the R. Q.'s obtained the first year.

TABLE III

EFFECT OF TEMPERATURE ON THE RESPIRATORY ACTIVITY OF NORTHERN RED OAK ACORNS

DATE	TEMPERATURE OF STORAGE	TEMPERATURE OF EXPERIMENT	GAS	NUMBER OF OBSERVATIONS	MEAN VOLUME OF GAS PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
Northern red oak								
	°C.	°C.			mm.³	mm.³	%	
1–15–37	0	0	CO_2	31	20.50	1.69	8.23	0.2693
			O_2	29	76.12	4.38	5.75	
1–16–37	0	5	CO_2	27	19.48	2.54	13.03	0.3013
			O_2	27	64.66	3.81	5.90	
1–17–37	10	10	CO_2	30	29.10	2.52	8.66	0.3181
			O_2	30	91.48	7.22	7.89	
1–18–37	10	15	CO_2	30	54.52	3.74	6.85	0.3759
			O_2	30	145.32	7.96	5.48	
2– 6–37	10	20	CO_2	32	65.58	5.60	8.54	0.4888
			O_2	32	134.16	6.34	4.72	
1–20–37	10	24	CO_2	30	88.18	6.90	7.82	0.5208
			O_2	30	142.04	10.42	7.34	
1–21–37	10	30	CO_2	25	117.94	10.58	8.97	0.5442
			O_2	25	216.72	17.71	8.17	

Figures 6 and 7 have been constructed from the data presented in table VI. Figure 6 shows the respiratory activity of northern red oak acorns at different temperatures at various times during after-ripening while figure 7 shows the progress of respiratory activity at each storage temperature throughout the period of after-ripening. It is clearly shown in figures 6 and 7 that the R. Q. of northern red oak acorns decreases more rapidly during a period of storage at 2.5° C. than it does at the other temperatures.

CATALASE ACTIVITY

An experiment was performed to trace the catalase activity of both species through the storage period. Measurements were made at 25° C. of the

catalase activity of acorns from all of the storage lots at the time of storage and at the time of removal from storage. Another set of measurements was made of white oak acorns and northern red oak acorns which had been allowed a 24-hour germination period at 25° C. prior to preparing the material for measurement.

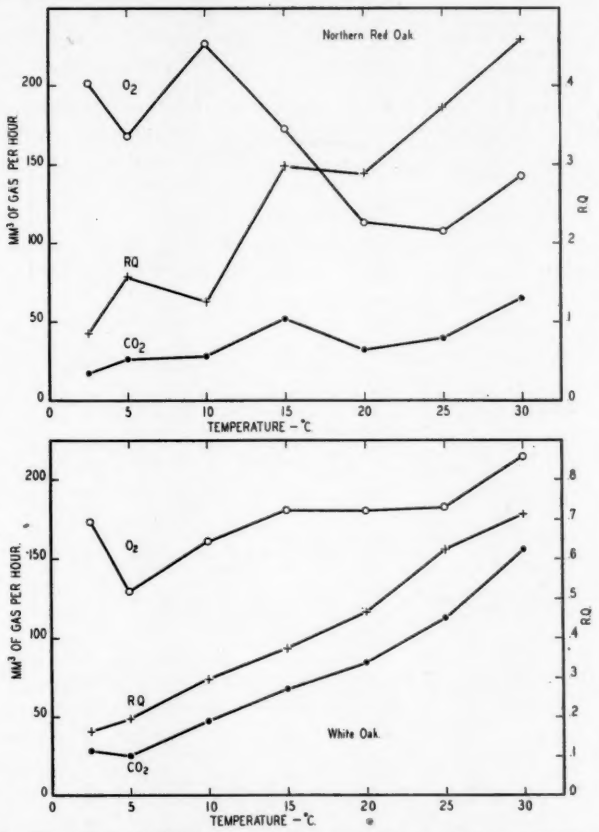

FIG. 5. The effect of different temperatures on the respiratory activity of northern red oak and white oak acorns after storage at 2.5° C. in 1937–1938.

The catalase activity of both species of acorns at 2.5° C. decreased slightly during the period of storage, although the activity of the white oak acorns was about three times that of the northern red oak acorns (figure 8A). The northern red oak acorns stored at 12.5°, 15.0°, and 17.5° C. showed an increase in activity during storage. The rate of catalase activity was lower in the lot stored at 15.0° than in the lots stored at 12.5° and 17.5° C.

The white oak acorns stored at 2.5° and allowed a 24-hour germination period at 25° C. showed an increase in catalase activity during the first half of the period of storage which was followed by a slight decrease in the latter

TABLE IV

NORTHERN RED OAK AND WHITE OAK ACORN RESPIRATION MEASURED AT 2.5°, 5°, 10°, 15°, 20°, 25°, AND 30° C. AFTER STORAGE AT 2.5° C.

DATE	TEMPERATURE OF EXPERIMENT	GAS	NUMBER OF OBSERVATIONS	GAS PER GRAM DRY WEIGHT PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
			Northern red oak				
	° C.			mm.³	mm.³	%	
1- 7-38	2.5	CO_2	10	17.58	3.30	18.77	0.0871
		O_2	10	201.74	14.22	7.05	
1- 7-38	5	CO_2	10	26.64	3.64	13.66	0.1591
		O_2	10	167.42	25.24	15.08	
1- 7-38	10	CO_2	10	28.76	2.52	8.76	0.1270
		O_2	10	226.44	20.50	9.05	
1- 8-38	15	CO_2	10	51.52	19.40	37.66	0.2988
		O_2	10	172.46	20.50	11.89	
1- 9-38	20	CO_2	10	32.86	4.48	13.63	0.2891
		O_2	10	113.66	9.60	8.45	
1- 9-38	25	CO_2	10	39.96	8.14	20.37	0.3714
		O_2	10	107.60	13.08	12.16	
1-10-38	30	CO_2	10	65.64	15.70	23.92	0.4600
		O_2	10	142.70	24.70	17.31	
			White oak				
12-16-37	2.5	CO_2	10	28.42	4.06	14.29	0.1636
		O_2	10	173.68	9.72	5.60	
12-17-37	5	CO_2	10	25.40	3.88	15.28	0.1952
		O_2	10	130.14	10.94	8.41	
12-17-37	10	CO_2	10	47.74	5.10	10.68	0.2977
		O_2	10	160.34	6.10	3.80	
12-18-37	15	CO_2	10	67.72	5.34	7.89	0.3737
		O_2	10	181.22	16.92	9.34	
12-18-37	20	CO_2	10	84.38	9.40	11.14	0.4673
		O_2	10	180.56	15.32	8.48	
12-19-37	25	CO_2	10	113.34	6.60	5.82	0.6211
		O_2	10	182.48	9.34	5.12	
12-19-37	30	CO_2	10	152.88	11.54	7.55	0.7127
		O_2	10	214.52	10.20	4.75	

half. The northern red oak acorns subjected to the same temperature conditions showed a steady increase in catalase activity during the whole period of measurement (fig. 8B and table VII).

Lack of material prevented further catalase measurements at this time.

Germination tests

In 1936, 250 acorns of each species of oak were planted at the time of collection in a mixture of equal parts of sand and peat in a greenhouse to determine the percentage of germination. In 1937, 200 white acorns and 100 northern red oak acorns were planted in the same manner for the same purpose. The diurnal variation in temperature of the greenhouse was 55° to 90° F. (12.8° to 32.2° C.). The time of germination was arbitrarily chosen as the time when the plumule of the seedlings appeared at the surface of the ground.

The germination results indicate a great difference (figs. 9A and 10A) in temperature requirements for the germination of white oak acorns and the after-ripening and germination of northern red oak acorns. Within a period

TABLE V

Effect of storage temperature on the respiratory activity of northern red oak acorns. Respiratory measurements made at storage temperatures

Date	Storage temperature	Gas	Number of observations	Mean volume of gas per hour	Standard error of mean	Standard error of mean	Respiratory quotient
				Northern red oak			
	°C.			mm.³	mm.³	%	
1–15–37	0	CO_2	31	20.50	1.69	8.23	0.2693
		O_2	29	76.12	4.38	5.75	
1–17–37	10	CO_2	30	29.10	2.52	8.66	0.3181
		O_2	30	91.48	7.22	7.89	
1–19–37	20	CO_2	30	36.42	1.94	5.32	0.5101
		O_2	30	71.40	3.22	4.50	

of 39 days after collection (fig. 9A) all of the viable white oak acorns had germinated. Of the 250 white oak acorns, 221 (88.4 per cent.) germinated. The following year (fig. 10A) 167 of 200 (83.5 per cent.) white oak acorns proved viable and had germinated in 35 days after collection. Both seasons some of the white oak acorns had protruding radicles at the time of collection showing the extent of their ability to germinate very soon after they fall from the tree.

Only one northern red oak seedling appeared (fig. 9A) in 40 days after planting and in 130 days 20 seedlings (8 per cent.) had appeared. At the time the experiment ended (July 10, 1937) 44 per cent. of the acorns had germinated. The following year (fig. 10A) 48 days elapsed before a seedling appeared and at the end of the experiment (February 16, 1938) 12 per cent. of the lot had germinated. It is apparent that the greenhouse temperatures were too high for the proper after-ripening of the northern red acorns.

Plots of 88 northern red oak acorns were set out in a greenhouse in 1936 to show the effect of three different storage temperatures (0°, 10°, and 20° C.) on the time of germination. The material had been kept in storage for

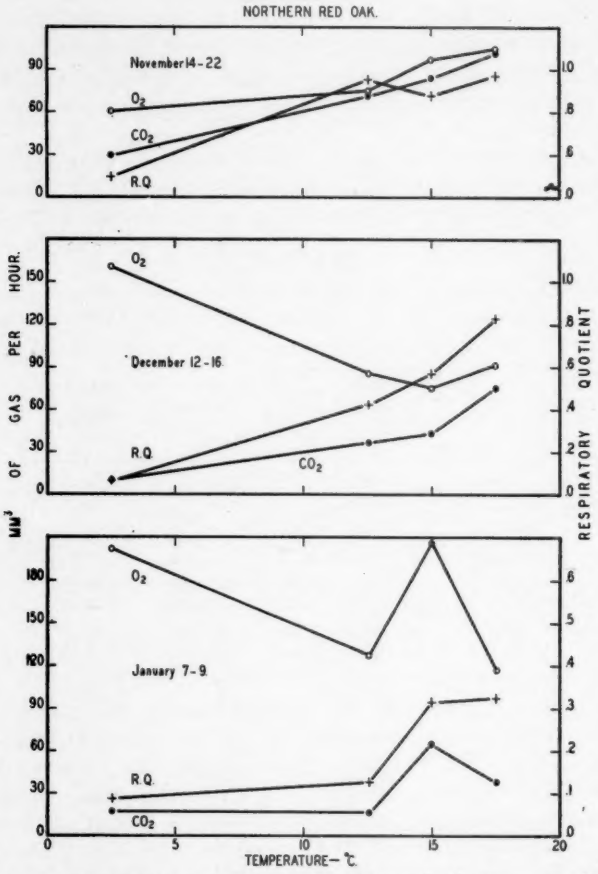

FIG. 6. Stages of respiratory activity of northern red oak acorns stored at different temperatures in 1937–1938. Measurements were made at the storage temperatures.

approximately two and one-half months. The following year (1937) 70, 79, 73, and 62 northern red oak acorns were set out under the same conditions except that they had been stored at 2.5°, 12.5°, 15.0°, and 17.5° C.

The 1936 germination tests on northern red oak acorns (fig. 9B) showed that in 30 days the material which had been stored at 0° C. had 83 per cent.,

TABLE VI

EFFECT OF STORAGE TEMPERATURES ON THE RESPIRATION OF NORTHERN RED OAK ACORNS DURING AFTER-RIPENING. MEASUREMENTS OF RESPIRATION WERE MADE AT THE STORAGE TEMPERATURES

DATE	GAS	NUMBER OF OBSERVATIONS	GAS PER GRAM DRY WEIGHT PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
			2.5° C.			
			*mm.*³	*mm.*³	%	
11–21–37	CO_2	18	29.94	3.62	12.09	0.4990
	O_2	18	60.00	5.20	8.67	
12–16–37	CO_2	10	9.86	1.94	19.68	0.0613
	O_2	10	160.88	11.98	7.45	
1– 7–38	CO_2	10	17.58	3.30	18.77	0.0871
	O_2	10	201.74	14.22	7.05	
			12.5° C.			
11–22–37	CO_2	18	71.14	5.78	8.12	0.9523
	O_2	18	74.70	4.72	6.32	
12–16–37	CO_2	10	36.78	4.58	12.45	0.4269
	O_2	10	86.16	11.32	13.14	
1– 8–38	CO_2	10	16.30	2.98	18.28	0.1272
	O_2	10	128.16	9.68	7.55	
			15° C.			
11–14–37	CO_2	18	83.94	4.58	5.46	0.8677
	O_2	18	96.74	5.46	5.64	
12–12–37	CO_2	10	43.18	3.82	8.85	0.5724
	O_2	10	75.44	6.10	8.09	
1– 8–38	CO_2	10	64.98	16.00	24.62	0.3153
	O_2	10	206.10	22.36	10.85	
			17.5° C.			
11–15–37	CO_2	18	101.48	8.84	8.71	0.9711
	O_2	18	104.50	8.52	8.15	
12–12–37	CO_2	10	75.88	7.90	10.41	0.8293
	O_2	10	91.50	9.54	10.43	
1– 9–38	CO_2	10	38.12	8.02	21.04	0.3254
	O_2	10	117.18	9.94	8.48	

that stored at 10° C. 93 per cent., and that stored at 20° C. only 6.8 per cent. germination. The following year the total percentages of germination were 68.6, 83.5, 69.9, and 43.6 for material which had been stored at 2.5°, 12.5°, 15.0°, and 17.5° C., respectively (fig. 10B). Both years the lowest storage temperatures were not favorable for the highest percentage of total ger-

mination nor did germination start first in these lots when placed under conditions favorable for germination. Northern red oak acorns stored at 10° and 12.5° C. started germinating sooner, germinated more rapidly and had a higher percentage of germination than acorns of the same species stored at other temperatures in these experiments.

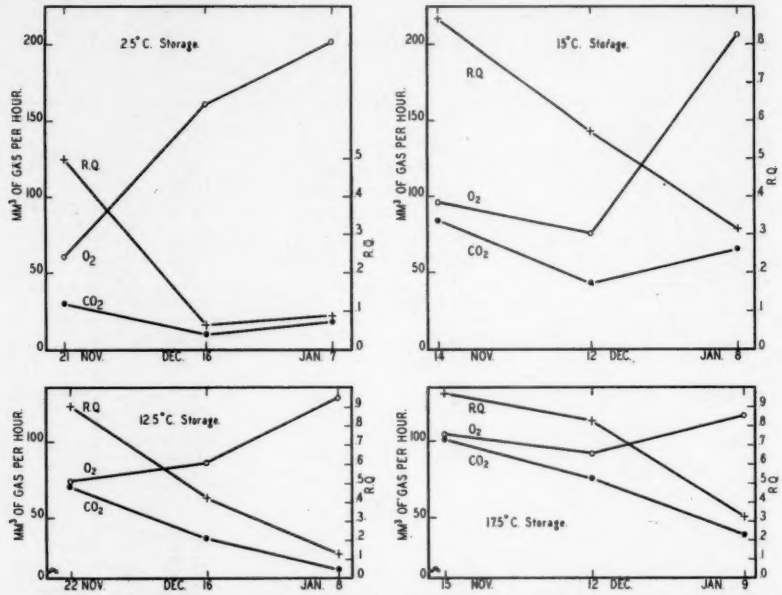

FIG. 7. The effect of storage temperatures on the respiratory activity of northern red oak acorns in 1937–1938. Measurements were made at the storage temperatures.

Discussion

TIME REQUIRED TO ADJUST ACORNS TO A NEW TEMPERATURE

The data presented in figure 1 indicate that at least 6 hours were required for the rate of respiration to become constant when the temperature of the acorns was increased from 10° to 20° C. The time required would probably have been longer if the fruit coats had not been removed. Evidently, overnight storage in a CO_2-free atmosphere at the experimental temperature allowed sufficient time for the establishment of a new equilibrium with respect to CO_2 exchange between the acorns and their environment. It is probable that any marked change in the environment of living material, such as removal of seed or fruit coats, changes in temperature, or in the concentration of the surrounding atmosphere, should be followed by a sufficient

time interval to allow establishment of physical and physiological equilibrium under the new conditions. The time interval required for any particular material can probably be determined only by experiment.

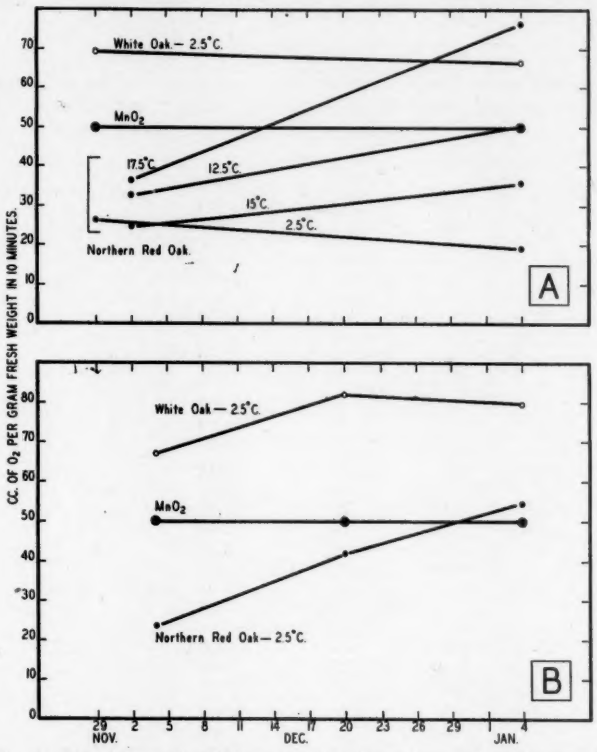

FIG. 8. A. The changes in catalase activity of northern red oak acorns during storage at different temperatures in 1937–1938. Measurements were made at 25° C. immediately after removal from storage.

B. The changes in catalase activity of white oak and northern red oak acorns during storage at 2.5° C. in 1937–1938. Measurements were made at 25° C. after a 24-hour germination period at that temperature.

COMPARISON OF RESPIRATION IN TWO SPECIES OF OAK ACORNS

The production of CO_2 by acorns of the two species when stored and measured at 0° and 2.5° C. was consistently low during the entire storage period, that of the northern red oak being the lower. During the storage period the O_2 consumption of the white oak acorns increased to a very high level which was maintained during the latter part of the period. The O_2

consumption of the northern red oak acorns was lower than that of the white oak acorns at the beginning of the season. It increased during the period of storage and in 1937–1938 reached a higher level during midwinter than was reached by white oak acorns stored at the same temperature.

TABLE VII

EFFECT OF STORAGE TEMPERATURE AND GERMINATING CONDITIONS ON THE CATALASE ACTIVITY OF WHITE OAK AND NORTHERN RED OAK ACORNS AT 25° C. (ADJUSTED TO MnO_2 CONTROL). WO = WHITE OAK; NRO = NORTHERN RED OAK

DATE	STORAGE TEMPERATURE	MATERIAL	NUMBER OF OBSERVATIONS	O_2 PRODUCED IN 10 MINUTES	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN
Without germination period						
	°C.			*ml.*	*ml.*	*%*
11–29–37	2.5	WO	9	69.33	2.03	2.92
	2.5	NRO	9	26.40	0.97	3.69
		MnO_2	4	50.00	0.54	1.08
12– 2–37	12.5	NRO	5	32.70	1.20	3.67
	15.0	NRO	5	24.50	1.03	4.18
	17.5	NRO	5	36.33	1.50	4.20
1– 4–38	2.5	WO	5	66.45	0.95	1.43
	2.5	NRO	5	19.15	0.88	4.57
	12.5	NRO	5	50.26	2.23	4.23
	15.0	NRO	5	35.90	1.13	3.13
	17.5	NRO	5	76.15	1.35	1.77
		MnO_2	3	50.00	0.85	0.68
With germination period						
12– 4–37	2.5	WO	5	66.90	1.03	1.53
	2.5	NRO	5	23.50	1.25	5.32
		MnO_2	4	50.00	0.54	1.08
12–20–37	2.5	WO	5	82.00	1.95	2.38
	2.5	NRO	5	42.08	1.78	4.22
		MnO_2	4	50.00	1.44	2.88
1– 4–38	2.5	WO	5	79.73	1.83	2.29
	2.5	NRO	5	54.30	0.88	1.61
		MnO_2	3	50.00	0.85	0.68

The respiratory quotients (R. Q.'s) are somewhat similar in the two species both being very low and both showing a decrease during the storage period. A difference in R. Q.'s might have been expected since KORSTIAN (**6**) found northern red oak acorns to contain a much higher percentage of fats than white oak acorns. The actual decreases in fat content were rather similar, however, in the two species. Apparently the same sort of metabolic activities occur in acorns of both species. The fact that the CO_2 production was relatively constant during the entire storage period suggests that the

rate of cellular respiration may have been approximately constant. The increased O_2 consumption may have resulted from an increase in rate of conversion of fats into carbohydrates, a process which requires large quantities of oxygen and which may even result in an increase in dry weight.

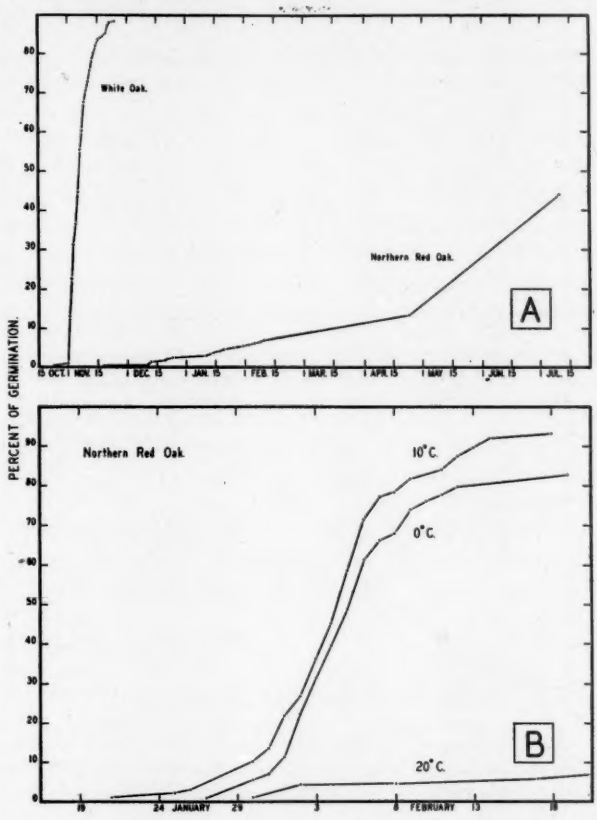

FIG. 9. A. The difference in germination of white oak and northern red oak acorns which have been planted in a greenhouse at the time of collection in 1936–1937.

B. The effect of storage temperatures on the germination of northern red oak acorns in 1936–1937.

KORSTIAN (6) reported that during the storage period from November to April the fat content of both red and white oak acorns decreased while the carbohydrate content increased. The low R. Q.'s found in the present experiments are such as would be expected if fats are being converted to carbohydrates rather than being completely oxidized. MILLER (9) in his

summary of the literature dealing with chemical changes in germination of oily seeds mentions a number of instances where the fat content decreased while the carbohydrate content increased.

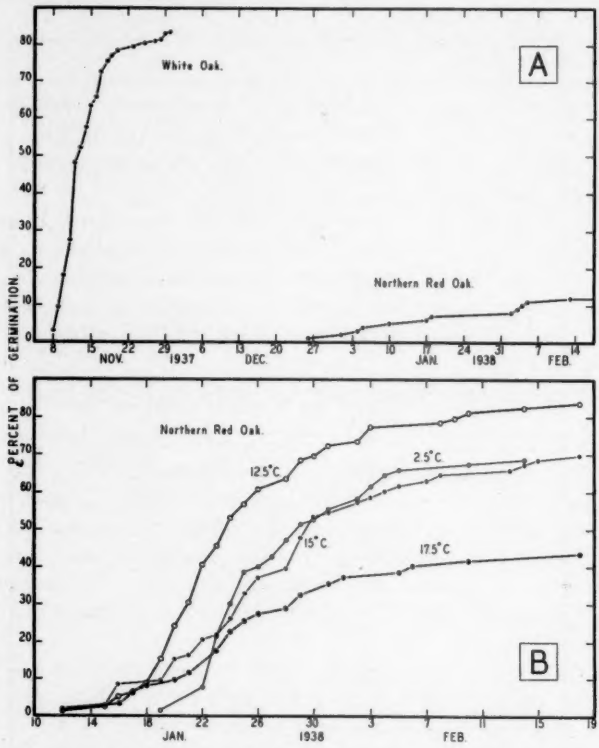

FIG. 10. A. The difference in germination of white oak and northern red oak acorns which have been planted in a greenhouse at the time of collection in 1937–1938.

B. The effect of storage temperatures on the germination of northern red oak acorns in 1937–1938.

HUTCHINS (5) found that oil from acorns of *Quercus palustris* had the following composition:

	PERCENTAGE
Glycerides of linoleic acid	27.92
Glycerides of oleic acid	57.01
Glycerides of saturated acids	15.01

The following formulae show how any oleic acid present in acorns may be oxidized:

$$2(C_{18}H_{34}O_2) + 17\ O_2 + 2H_2O \rightarrow 6(C_6H_{12}O_6) \quad \text{(Complete conversion of oleic acid to glucose)}$$

$$\text{R. Q.} = 0.000$$

$$2(C_{18}H_{34}O_2) + 51\ O_2 \rightarrow 36\ CO_2 + 34\ H_2O \quad \text{(Complete oxidation of oleic acid)}$$

$$\text{R. Q.} = \frac{36}{51} = 0.706$$

These theoretical equations show that the R. Q. of oleic acid may extend over a range of 0.000 to 0.706 depending on the proportion converted to stored carbohydrates and the proportion oxidized following digestion. The behavior of oleic acid is representative of what might be expected of other fatty acids present in acorns.

There is a possible indication, shown by the constant CO_2 production (fig. 3), that the oxidation of the carbohydrates proceeded at a constant rate throughout the whole period of storage but that the conversion of fatty acids to carbohydrates increased rapidly between November 21 and December 16, 1937, where it reached an equilibrium which existed until the end of the storage period.

Effects of temperature on respiration of acorns

The result of studies of the rates of respiration at different temperatures are not entirely consistent, especially for northern red oak. Both species showed a consistent increase in CO_2 production and in R. Q. with increasing temperature both years (figs. 4A and 5). The O_2 consumption of white oak acorns did not change materially with a change in temperature. The O_2 consumption of northern red oak acorns increased with temperature in 1936–1937, but in 1937–1938 it decreased. No explanation of this variation can be offered.

The consistently increasing R. Q.'s of both species indicate that possibly as the temperature increases there is a change from carbohydrate accumulation, as a result of conversion of fatty acids in excess of oxidation of carbohydrates, to a greater rate of oxidation of carbohydrates.

Effect of storage temperatures on R. Q. and rate of respiration of northern red oak acorns

It is found that the lower storage temperatures are accompanied by lower R.Q.'s and higher percentages of germination when planted at greenhouse temperatures (figs. 4B, 7, 9B, and 10). Figure 7 is possibly indicative of a more efficient accumulation of carbohydrates at the 2.5° and 12.5° C. storage temperatures. It is possible that the material stored at 2.5° C. reached an efficient stage of carbohydrate accumulation earlier and was apparently subjected to it for a longer period than the other 3 lots stored at different temperatures. The germination results of this lot were almost the same as the lot which was stored at 15° C. The lot stored at 12.5° reached this efficient

stage of accumulation at the end of the storage period and then had the highest percentage and quickest germination of the 4 lots. The lots of material which were stored at 15° and 17.5° C. did not reach an R.Q. lower than 0.310 while the R.Q.'s of the lots stored at 2.5° and 12.5° C. were as low as 0.061 and 0.127 (table VI). It seems possible that the accumulation of carbohydrates varies inversely with the storage temperature in these experiments but that the best physiological balance has been obtained by storage of the acorns at 10° or 12.5° C. There was a noticeable lag in the germination of the lot stored at 2.5° C. (fig. 10B) which may have been caused by a secondary dormancy. Further investigation of this point is desirable.

Apparently, northern red oak acorns must be subjected to conditions allowing a period of carbohydrate accumulation before they become properly after-ripened. The successful use of Warburg respirometers for an indication of the stage of after-ripening will depend on the length of period necessary for after-ripening, which in turn will depend on the temperature at which the material is stored. From the present study there is an indication that after-ripening is completed in 6 weeks of storage at 12.5° C. while there is a possibility that 3 weeks of storage at 2.5° C. is sufficient for this process (fig. 7). This point could be confirmed by removing the acorns from low temperature storage (2.5° C.) to conditions favorable for germination as soon as the R.Q. of the material has reached a low level (0.1 to 0.2). It has been pointed out by KORSTIAN (6) that after-ripening was hastened by temperatures of 50° to 65° F. but while these temperatures may furnish conditions for optimum germination it is posible that lower temperatures are more favorable for quicker after-ripening.

Undoubtedly, the best measurements of respiration are those which include the consumption or uptake of O_2 and the production of CO_2. There are methods of measuring respiration however, which are concerned with the measurement of the exchange of only one of these gases. It has been found in this study that the production of CO_2 is more constant than the consumption of O_2 and, therefore, is less affected by chemical transformations of food in the respiring material. Where both after-ripening and respiration are concerned, a measurement of the CO_2 output does not offer as accurate an indication of any chemical changes of food as a measurement of the O_2 consumption.

CATALASE ACTIVITY

BALDWIN (3) suggested the following catalase test of seed viability as having been found applicable to several coniferous species:

$$\text{When the quotient} \frac{O_2 \text{ released from } H_2O_2 \text{ by catalase of stimulated seed}}{O_2 \text{ released from } H_2O_2 \text{ by catalase of resting seed}}$$

is greater than unity the seeds are considered viable.

In the present study such an indication of viability was found to be of little value for northern red oak acorns, since catalase activity is not directly related to after-ripening and the latter seems to be the controlling factor affecting germination. Viable northern red oak acorns will not germinate unles they are properly after-ripened. Tests based on catalase activity indicated that neither white oak nor northern red oak acorns were viable at the beginning of the experiment, yet later by the same test they were viable.

Catalase activity does not offer a measure of after-ripening (fig. 8A) since the lot of acorns stored at 17.5° C. shows the greatest catalase activity at the end of the storage period and yet has the lowest percentage of germination apparently because of incomplete or insufficient after-ripening. AFANASIEV (1) also found that catalase activity was apparently independent of the degree of after-ripening.

GERMINATION

AIKMAN (2) found that the germination of northern red oak acorns was markedly slower than the germination of white oak, black oak, and bur oak although 94 per cent. germination was obtained after 138 days. This percentage was obtained on a basis of 20 acorns of each species planted directly in a greenhouse where the temperature varied from 55° to 90° F. The results obtained in the present study do not agree with the results obtained by AIKMAN. In 1936–1937, 250 northern red oak acorns planted directly in a greenhouse having approximately the same temperature range as that used by AIKMAN gave 8 per cent. germination; and 100 acorns of the same species planted the following year gave 12 per cent. germination, in about the same time interval used by AIKMAN.

KORSTIAN (6) found that an average night temperature of 50° and 65° F. during the day gave the most satisfactory results with red, black, white, scarlet, and chestnut oak acorns when both promptness of germination and total germination were considered. Records of diurnal variations of greenhouse temperatures were not kept in the present study.

SUGGESTIONS FOR FURTHER STUDY

The measurement of respiration during the period of storage of white oak and some acorns of the red or black oak group stored at 0°, 2.5°, 5°, 7.5°, 10°, 12.5°, and 15° C., supplemented with germination tests, should definitely indicate the best storage temperature for the rate of after-ripening desired. At the time when the lots show an R.Q. near 0.3, germination plots should be set out weekly or bi-weekly thereafter until the R.Q. remains constant for at least two weks. The results of these germination tests will establish the limits of use of the R.Q. as an indicator of after-ripening.

Summary

Measurements of respiration, catalase activity and germination of white oak (*Quercus alba* L.) and northern red oak [*Quercus borealis* var. *maxima*

(Marsh.) Ashe] acorns which had been stored at 0° and 2.5° C. were made in order to obtain an indication of the physiological differences between the two species of acorns during the over-wintering period. Measurements of respiration and germination tests of northern red oak acorns which had been stored at 0°, 2.5°, 10°, 12.5°, 15°, 17.5°, and 20° C. were made during the overwintering period to determine the physiological changes and the progress of after-ripening of the acorns at these different temperatures.

1. Northern red oak acorns approach constant respiratory activity after 6 hours of exposure to a new constant temperature.

2. Although the rates of gas exchange may differ slightly the physiological activity of both species of acorns is very similar as indicated by similar respiratory quotients when the acorns are stored at 0° and 2.5° C.

3. The CO_2 production of both species of acorns remained constant during overwinter storage at 2.5° C. while the O_2 consumption of white oak acorns increased markedly after the first 3 weeks of storage and the O_2 consumption of northern red oak acorns increased rapidly during the first 3 weeks of storage. Because of this behavior the respiratory quotients were lowered from about 0.50 to 0.09 and 0.16 respectively where they remained constant during the last 3 weeks of storage, possibly indicating a period of carbohydrate accumulation.

4. The respiratory quotients of both species of acorns generally increased with temperature when removed from storage at 2.5° C., suggesting a greater use of carbohydrates at the higher temperatures.

5. The respiratory quotient of northern red oak acorns stored at temperatures of 2.5°, 12.5°, 15°, and 17.5° C. decreased more rapidly at the lowest storage temperature. This behavior suggests that possibly carbohydrate acumulation started earlier and proceeded at a greater rate at the lower storage temperatures.

6. Measurements of catalase activity did not materially aid in interpreting the progress of after-ripening or the potential germinating ability of the acorns.

7. Germination tests in the greenhouse of northern red oak acorns which had been previously stored at 0°, 2.5°, 10°, 12.5°, 15°, 17.5°, and 20° C. for two months showed that storage temperatures of 10° and 12.5° C. were best for the germination of northern red oak acorns.

8. The northern red oak acorns which showed the highest percentage of germination had a respiratory quotient lower than 0.3 in storage.

The writer expresses his gratitude for the helpful criticism of Dr. PAUL J. KRAMER during the course of this study and for his material assistance in the preparation of this paper. Indebtedness is acknowledged to Professor FRANCIS X. SCHUMACHER for suggestions on the sampling procedure and the

statistical treatment of the data, and to Dr. CLARENCE F. KORSTIAN for advice on the treatment of acorns for storage.

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DISTRIBUTION OF NITROGENOUS FRACTIONS, SUGARS, AND OTHER SUBSTANCES IN ANANAS GROWN IN DARKNESS VERSUS DAYLIGHT[1]

C. P. Sideris, B. H. Krauss, and H. Y. Young

(WITH FOURTEEN FIGURES)

Introduction

The object of the present studies is to report and compare data on the nitrogen metabolism of two sets of plants, both of which were in an early stage of fruiting at the time of shifting some of them to continual darkness, and leaving the remainder under the seasonal light conditions of a glass house. The former set was kept in a darkened box for four months during which time there was little expansion of new leaf tissue since, in the pineapple, leaf growth practically ceases after sexual differentiation. The small fruits already formed at the start of the experiment increased in size and ripened. Since the period of darkness was quite long the analytical results obtained were decidedly different for the two sets of plants.

The literature on nitrogen metabolism has been reviewed in the first paper (**6**) of this series. Since that time a paper by Vickery *et al.* (**9**) has appeared which contrasts the physiological behavior of excised matured tobacco leaves in light and darkness. Murneek, in a recent paper (**3**), contrasted the relationships between carbohydrates and nitrogen in connection with photoperiodism. Burkholder (**1**) has reviewed very extensively the rôle of light in the life of plants. The reviews on nitrogen metabolism by Nightingale (**4**) and McKee (**2**) practically bring our knowledge of experimental work on the physiological rôle of nitrogen in plant nutrition up to date.

Experimental methods

Two sister shoots of uniform weight and appearance were removed from each of several mother plants which were growing in the field. Each of the shoots were at the red bud stage; that is, when the fruit bud was visible between the apical leaves of the stem but had not yet emerged completely. One of each set of the shoots was placed in a group grown under seasonal greenhouse conditions and the other in a group grown under conditions of continual darkness. The shoots in both groups however, were grown for the first month in the greenhouse under identical quantities and qualities of light to allow them to produce good root systems and new leaves. At the end of this period, *i.e.*, on February 15, 1935, the plants of one of the groups were transferred to a box, ten feet long, four feet wide, and four feet high; venti-

[1] Published with the approval of the Director as Technical Paper no. 114 of the Pineapple Experiment Station, University of Hawaii.

lated with two fans, one drawing air inwards and the other outwards, and kept dark at all times except during the renewal of nutrient solutions.

The nitrate-complete nutrient solution was changed weekly, at which time the volumes of absorbed and evaporated water were determined. LIVINGSTON atmometers were set up in the darkened box and in the greenhouse to obtain records of the rate of water evaporation under the two environments. Constant temperature records obtained in both environments fluctuated between 70° and 85° F. The temperature in the greenhouse was about 5 degrees higher between the hours of 11 A.M. and 4 P.M. than in the box. At all other times the temperature values were approximately the same.

The plants were kept in their respective environments until the fruits matured. The fruits of the plants kept in the darkened box matured and ripened about one month earlier than those in the greenhouse. Consequently, the former plants were harvested on June 15, 1935, *i.e.*, after four months of continuous darkness. The plants kept in the greenhouse and exposed to light were harvested on July 15, 1935. The whole plant was harvested and its different organs were cut into as many sections as were deemed necessary for the proper interpretation of the physiological rôle of different plant organs and tissues in the metabolism of the plants under the conditions of the two experimental environments. The different plant sections were made in accordance with our previously described plan (7) which is shown for convenience in figure 1. They were prepared and analyzed with the technique and methods described in former publications (**6, 7, 8**).

The leaves, after detachment from the stem, were segregated into two lots. Lot I comprised the thoroughly mature and active leaves of groups C and D which had probably attained their maximum growth at the beginning of the experiment. Lot II comprised the younger leaves of the plant, *i.e.*, those belonging to groups E and F of fruit-bearing plants, as indicated in a former paper (7). The latter leaves had not completed growth at the beginning of the experiment but, at the time of harvest, they were thoroughly developed and mature. The terminal chlorophyllous sections, 4 and 5, of the leaves of groups (C and D, E and F) of the plants kept in darkness showed symptoms of chlorophyll breakdown and tissue dehydration and were considered dead at harvest. The latter were, of course, analyzed as separate plant fractions. The stem was separated into a basal section, comprising the region on which the bases of the leaves of groups B and C were attached, and into an apical or upper section to which the bases of the leaves of D and E groups were attached. The peduncle was separated from the fruit and stem.

At harvest time the crown, or apical vegetative outgrowth, was detached from the fruit and the latter dissected, separating the flesh from the shell tissues as in figure 1. The stem and leaf tissues of the crown were analyzed as a single unit.

FIG. 1. Longitudinal section of a pineapple plant at fruiting stage showing the stem and its two sections (A–C) and (D–E); peduncle (p), the flesh (f), core (c), shell (s), and crown of the fruit; the (B), (C), (D), and (E) groups of the leaves, and the shoot.

Results

PLANT AND FRUIT WEIGHTS

Corresponding plants in the two lots were sisters and of about the same weight at the beginning of the experiment. The plants grown in darkness did not gain as much in weight as those grown in light, as will be seen by observation of figure 2. In fact, the former plants actually lost weight as a result of the death of the terminal portions of the older leaves. The fruits of the lot of plants grown in light weighed about twice as much as those of the lot grown in darkness. The crowns of the fruits of the former series weighed four times as much as those of the latter. The longer growth period and exposure to light were probably responsible for the greater amounts of plant and fruit tissue produced by the plants grown in the greenhouse.

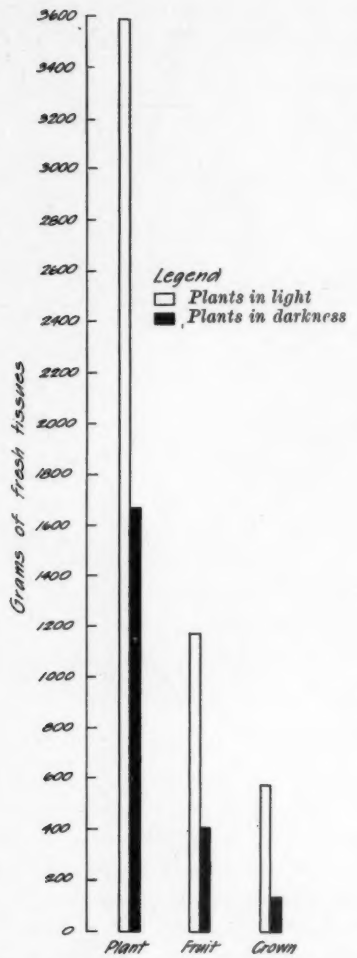

FIG. 2. Grams of fresh weight of plants, fruits, and crowns produced by two sets of pineapple sister shoots; one grown for 5 months in light, and the other for 4 months in darkness.

WATER ABSORPTION AND EVAPORATION

The volume of water absorbed by the two groups of plants was different, being greater for the plants exposed to light and less for those kept in darkness, as shown in figure 3. The relative degree of water evaporation, as determined with calibrated LIVINGSTON atmometers, varied slightly under

the two different environments, as shown in figure 4. In figure 5, where the data on water absorption are presented on a percentage basis, it will be seen that the plants exposed to sunlight absorbed approximately three to four times more water than those kept in darkness. The amounts of water evaporated by atmometers, however, under the two different environments were

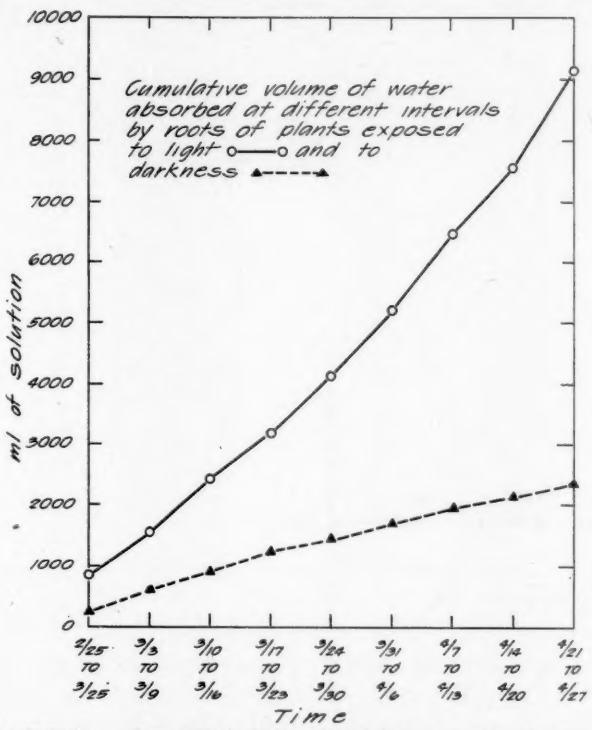

FIG. 3. Milliliters of nutrient solution absorbed by two sets of pineapple sister shoots; one grown in greenhouse light, and the other in darkness.

only slightly different, as the atmometer in the greenhouse evaporated only about 1.2 times more water than the one in the darkened box. The greater amounts of water absorbed by the plants in the greenhouse may be accredited in part therefore, to conditions more favorable for photosynthesis and transpiration Moreover, the plants grown in the greenhouse had more and better roots than those in the darkened box. This was not unexpected with the greater production of carbohydrate materials which in many cases have been found to be essential for root formation and functioning. The smaller

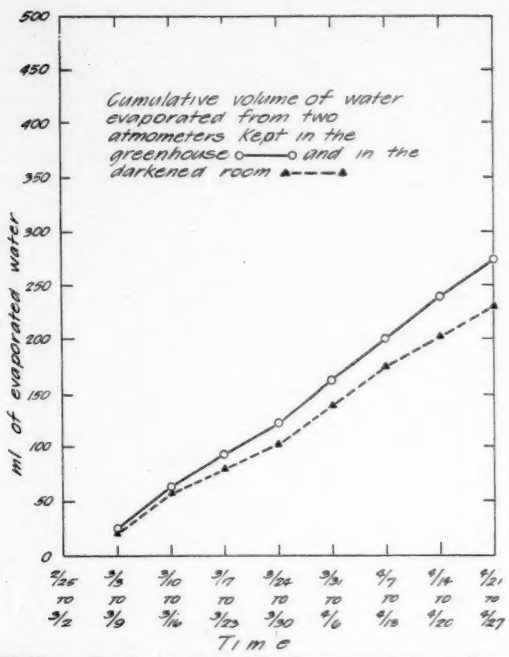

FIG. 4. Milliliters of water evaporated by LIVINGSTON atmometers; one kept in the greenhouse, and the other in the darkened box.

amounts of water absorbed by the plants in the darkened box were chiefly due to decreased growth.

TOTAL SOLIDS OF FRUITS

The distribution of total solids, determined refractometrically, is reported in figure 6 which shows that the percentage of total solids was greater in the fruit tissues of the plants exposed to light than in the tissues of those kept in darkness. In both groups, the tissues of the flesh contained slightly greater amounts of solids than those of the shell. This difference may not be of any physiological significance, because the tissues of the flesh accumulate, as a general rule, greater quantities of such solids as sugars than do those of the shell.

ACIDITY OF FRUITS

The fruits produced by both lots of plants varied considerably in acidity. The acid of the sap was titrated againt 0.1 N NaOH and is reported as per cent. citric acid. The amounts of titratable acidity as citric acid in the various sections of the fruits are presented in table I and figure 7.

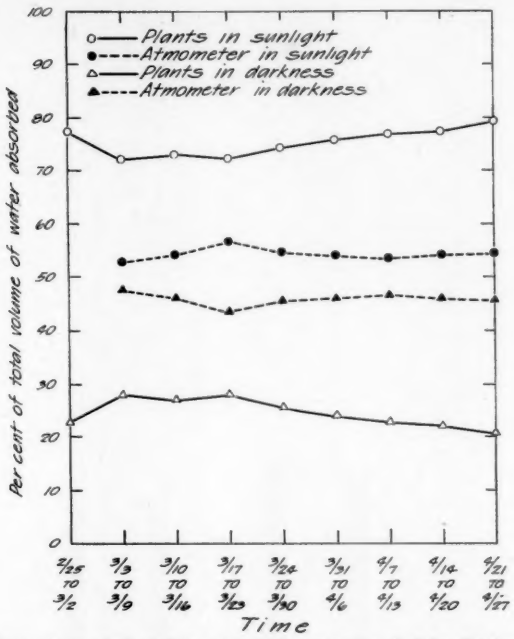

FIG. 5. Percentage of the total volume of water absorbed by the two sets of plants and also of that evaporated by the atmometers under the two different environmental conditions.

TABLE I

INITIAL AND FINAL PLANT WEIGHTS, FRUIT WEIGHTS, TITRATABLE ACIDITY, AND TOTAL SOLUBLE SOLIDS CONTENT OF TWO LOTS OF PLANTS, ONE EXPOSED TO LIGHT AND THE OTHER KEPT IN DARKNESS FROM AN EARLY STAGE OF FRUIT DEVELOPMENT

ITEMS	SERIES OF PLANTS	
	IN LIGHT	IN DARKNESS
Experiment started	2/15/35	2/15/35
Experiment harvested	7/13/35	6/17/35
Initial total weight, gm.	1900	1900
Final total weight, gm.	3585	1657
Final fruit weight, gm.	1175	406
Final crown weight, gm.	565	126
Titratable acidity* of shell base	1.15%	0.38%
Titratable acidity of shell apex	1.20%	0.34%
Titratable acidity of flesh base	0.90%	0.75%
Titratable acidity of flesh apex	0.95%	0.64%
Total soluble solids† of shell base	10.40%	3.20%
Total soluble solids of shell apex	9.80%	3.20%
Total soluble solids of flesh base	12.20%	3.80%
Total soluble solids of flesh apex	11.00%	4.00%

* Reported as per cent. citric acid.
† Determined refractometrically.

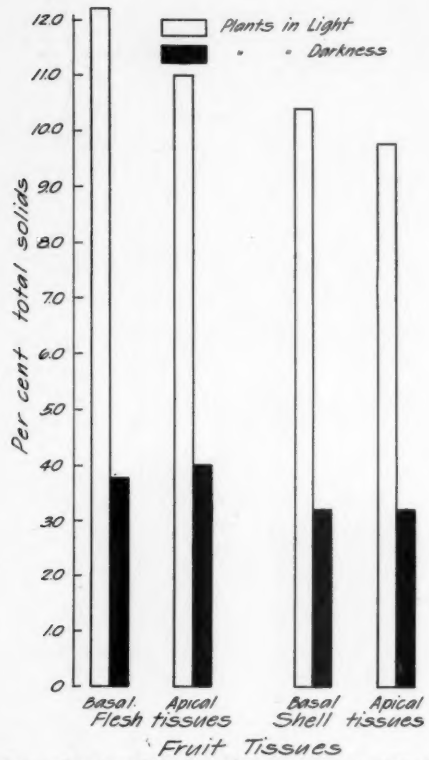

FIG. 6. Percentage of total solids, determined refractometrically, of the basal and apical tissues of the flesh and shell of fruits of pineapple sister shoots either exposed to greenhouse light or kept in darkness.

The data show that total acids in the flesh and shell tissues of the fruits of the plants exposed to light were considerably greater than those of corresponding sections of the fruits of the plants grown in darkness.

DISTRIBUTION OF NITROGENOUS FRACTIONS

The data for the distribution of different fractions of nitrogen in the various sections of the leaves of groups C–D and E–F, and of the stem and fruit of plants exposed either to light or kept in darkness are presented in tables II and III and in figures 9 to 11.

Reasons for the segregation of leaves and stem of pineapple plants into groups of different chronological and physiological ages, and for their sectioning into homogeneous lots, were presented and amply discussed in a former publication (7).

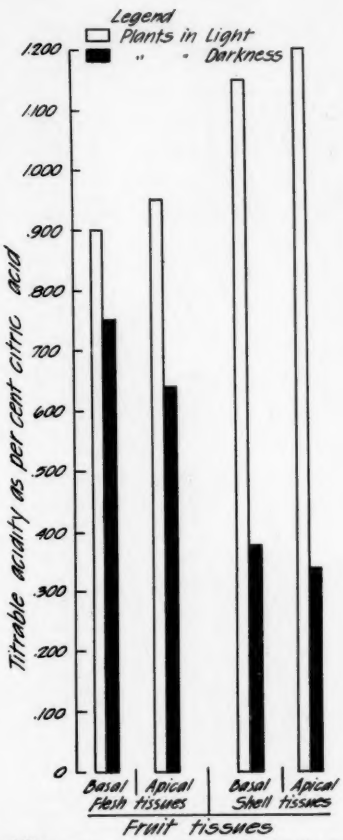

FIG. 7. Titratable acidity, as per cent. citric acid, of the basal and apical tissues of the flesh and shell of fruits of pineapple sister shoots either exposed to greenhouse light or kept in darkness.

The data for the mature leaf groups C and D of the plants kept in light and in darkness, as presented in figures 8-A and 8-B, show the following conditions:

1. Ammonium was found in very small quantities, while nitrate was present in considerably greater amounts in the basal non-chlorophyllous sections.

2. The quantities of glutamine were generally small. Those of asparagine were many times greater.

3. The quantities of mono-amino and basic nitrogen fractions were great in both the plants kept in light and in darkness. The quantities in the plants

TABLE II

DISTRIBUTION OF DIFFERENT FRACTIONS OF INORGANIC, SOLUBLE ORGANIC, AND INSOLUBLE ORGANIC NITROGEN IN THE TISSUES OF THE LEAVES, STEM, FRUIT, AND ROOTS OF *Ananas comosus* (L.) MERR. GROWN IN NITRATE SOLUTION CULTURES AND UNDER SEASONAL GREENHOUSE LIGHT CONDITIONS FOR FIVE MONTHS

Plant sections	Milligrams per gram of fresh tissue									
	Inorganic N		Soluble organic nitrogen				Insoluble organic nitrogen			
	Ammonium	Nitrate	Glutamine	Asparagine	Monoamino	Basic	Humin	Amide	Monoamino	Basic
Leaves	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Mature										
C1 + D1 (Base)	0.0051	0.0169	0.0088	0.1010	0.4262	0.3684	0.1300	0.0689	0.2129	0.0777
C2 + C3 + D2 + D3	0.0048	0.0060	0.0116	0.0896	0.3962	0.4235	0.1794	0.0561	0.3491	0.0648
C4 + D4	0.0052	0.0068	0.0166	0.1666	0.6062	0.6930	0.2608	0.0861	0.7478	0.0610
C5 + D5 (Tip)	0.0079	0.0110	0.0195	0.2146	0.6382	0.7806	0.3850	0.1307	0.7880	0.1593
Active										
E1 + F1 (Base)	0.0073	0.0375	0.0124	0.1172	0.4139	0.4278	0.1663	0.0976	0.2713	0.0525
E2 + F2	0.0024	0.0060	0.0070	0.0708	0.3364	0.2682	0.1400	0.0393	0.3080	0.0467
E3 + F3	0.0075	0.0032	0.0144	0.1112	0.5806	0.6056	0.2275	0.0595	0.6510	0.1365
E4 + F4	0.0080	0.0072	0.0191	0.2306	0.5567	0.6930	0.2975	0.0695	0.8170	0.1960
E5 + F5 (Tip)	0.0138	0.0078	0.0216	0.1000	0.8080	0.8860	0.3180	0.0870	0.8840	0.1818
Stem										
A + C (Lower)	0.0034	0.1977	0.0218	0.0700	0.6790	0.6965	0.1575	0.0341	0.3045	0.0840
D + E (Upper)	0.0132	0.1500	0.0271	0.1170	0.4850	0.5600	0.0980	0.0328	0.3010	0.0420
Peduncle	0.0025	0.3572	0.0067	0.0416	0.3082	0.3255	0.0665	0.0441	0.1085	0.0315
Fruit										
Shell	0.0172	0.0032	0.0170	0.1136	0.2302	0.3010	0.1365	0.0444	0.2065	0.0560
Flesh	0.0064	0.0050	0.0141	0.0390	0.1653	0.2128	0.0672	0.0261	0.0854	0.0182
Crown	0.0086	0.0603	0.0190	0.1616	0.6162	0.6458	0.1575	0.0641	0.4102	0.0798
Roots	0.0072	0.1043	0.0281	0.1072	0.4084	0.3570	0.2135	0.0556	0.3255	0.0770

TABLE III

DISTRIBUTION OF DIFFERENT FRACTIONS OF INORGANIC, SOLUBLE ORGANIC AND INSOLUBLE ORGANIC NITROGEN IN THE TISSUES OF THE LEAVES, STEM, AND FRUIT OF *Ananas comosus* (L.) MERR. GROWN IN NITRATE SOLUTION CULTURES AND IN DARKNESS FOR FOUR MONTHS

Plant sections	Milligrams per gram of fresh tissue									
	Inorganic N		Soluble organic nitrogen				Insoluble organic N			
	Ammonium	Nitrate	Glutamine	Asparagine	Mono-amino	Basic	Humin	Amide	Mono-amino	Basic
Leaves	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Mature										
C1 + D1 (Base)	0.0024	0.0313	0.0079	0.0800	0.3324	0.3920	0.0735	0.0257	0.1624	0.0616
C2 + D2 (Trans.)	0.0020	0.0438	0.0069	0.0876	0.2390	0.2744	0.1128	0.0387	0.1792	0.0560
Active										
E1 + F1 (Base)	0.0027	0.0438	0.0072	0.2184	0.4228	0.4172	0.0638	0.0202	0.1504	0.0560
E2 + F2	0.0027	0.0141	0.0144	0.1664	0.5720	0.5796	0.0938	0.0209	0.1960	0.0648
E3 + F3 (Green)	0.0030	0.0047	0.0089	0.3074	0.6135	0.6468	0.1649	0.0371	0.2688	0.0924
Stem										
A + D (Lower)	0.0058	0.0727	0.0113	0.3160	0.6540	0.6468	0.1530	0.0429	0.2828	0.0896
E + F (Upper)	0.0172	0.0313	0.0621	0.9864	2.3572	1.8480	0.0666	0.0257	0.2184	0.0728
Peduncle	0.0086	0.1539	0.0419	0.6864	0.8468	0.6804	0.0534	0.0212	0.0840	0.0392
Fruit										
Shell	0.0057	0.0141	0.0225	0.2352	0.4928	0.4648	0.0802	0.0349	0.1792	0.0588
Flesh	0.0107	0.0078	0.0450	0.7492	1.0310	0.9184	0.0550	0.0259	0.1120	0.0420
Crown	0.0040	0.0063	0.0246	0.3336	0.7292	0.7280	0.0750	0.0327	0.1932	0.0616

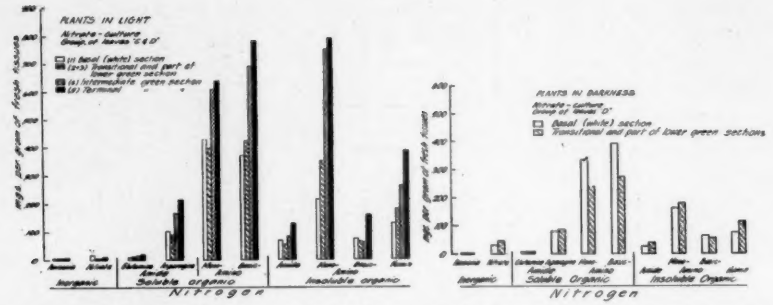

FIG. 8. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "C and D" groups of leaves of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "C and D" groups of leaves of pineapple sister shoots grown in solution cultures and kept in a darkened box for 4 months.

kept in light, however, were slightly greater than those in the plants kept in darkness.

4. The quantities of the various fractions of insoluble or protein nitrogen were generally greater in the plants kept in light than in those kept in darkness.

The data for the relatively young, but active, E and F leaf groups of the plants kept in light and in darkness, as presented in tables II and III and figures 9-A and 9-B, may be summarized as follows:

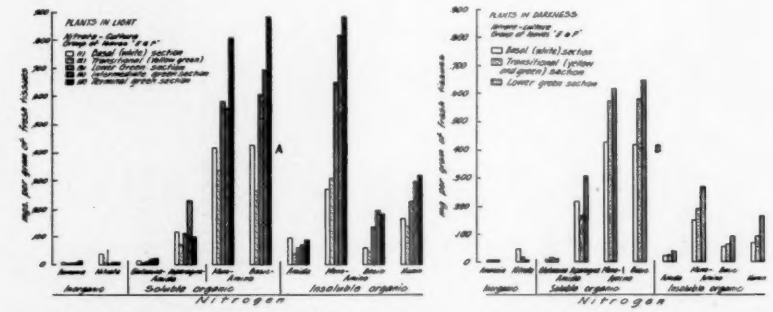

FIG. 9. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "E and F" groups of leaves of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "E and F" groups of leaves of pineapple sister shoots grown in solution cultures and kept in a darkened box for 4 months.

1. Ammonium occurred in traces. Nitrate was relatively abundant in the basal non-chlorophyllous and intermediate, or transitional, sections. In the chlorophyllous sections nitrate was present only in traces.

2. Glutamine was present in small amounts in the sections of the fruit from plants kept in light, but in considerably greater quantities in those of the fruit from the plants kept in darkness. Asparagine was present in appreciable quantities in the fruits from both the plants which had been kept in light and those kept in darkness. In the latter plants the quantities were greater than in the former.

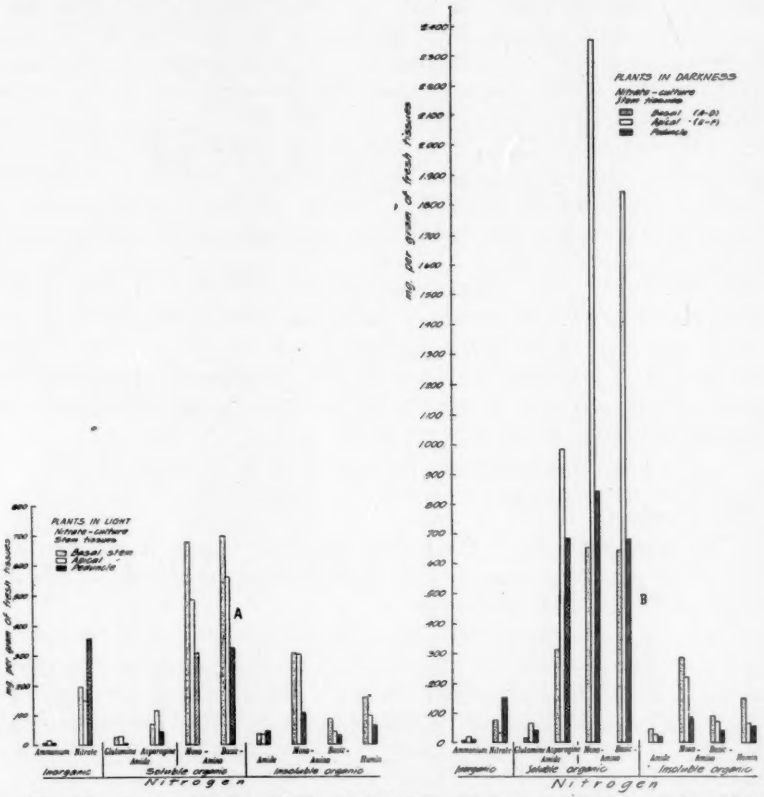

FIG. 10. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the lower and upper sections of the stem and peduncle of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the lower and upper sections of the stem and peduncle of pineapple sister shoots grown in solution cultures and kept in darkness for 4 months.

3. The difference between the quantities of soluble mono-amino and basic nitrogen found in the plants kept in light and those kept in darkness was very small and possibly of no significance.

4. All fractions of insoluble organic or protein nitrogen were generally in greater quantities in the plants kept in light than in those kept in darkness.

The data for the fully mature basal section and for the young and immature apical section of the stem and peduncle of both the plants kept in light and in darkness, presented in tables II and III and figures 10-A and 10-B, show the following:

1. Ammonium was present in mere traces. Nitrate was relatively abundant in all three sections of the stem of both the plants kept in light and in darkness, its quantities being considerably greater in the sections of the former than in the latter plants.

2. Glutamine was present in greater quantities in the mature basal stem section and in considerably less quantities in the apical and peduncle sections of the stem of plants kept in light than those of plants kept in darkness. Asparagine was present in very small quantities in the plants kept in light when compared with the plants kept in darkness in which the quantities were exceedingly great.

3. The quantities of soluble mono-amino and basic nitrogen fractions were exceedingly great in the apical stem section and peduncle of the plants kept in darkness. In the basal section of the stem, the difference in the quantities of soluble mono-amino and basic nitrogen in the plants kept in light and those kept in darkness was comparatively small.

4. The mono-amino insoluble organic nitrogen fraction was present in appreciably greater quantities in the apical section of the stem of the plants kept in darkness.

The data for the distribution of nitrogen in the flesh, shell and crown sections of the fruits of plants kept in light and in darkness, presented in figures 11-A and 11-B, show the following:

1. Ammonium was present in small quantities in the fruit of both groups of plants. In the fruits of plants kept in light, the quantities were slightly greater than in those kept in darkness. The quantities of nitrate were small in the shell and flesh sections of fruits of both groups of plants and in the crown of the plants kept in darkness. In the crown of the plants kept in light nitrate was found in appreciable quantities.

2. The quantities of glutamine, except in the flesh section of fruits of the plants kept in darkness where it was found in appreciable amounts, was small in all other fruit sections of both groups of plants. Asparagine was present in very large amounts in all three sections of fruit from both groups of plants, although in greater quantities in the flesh of the fruit from the

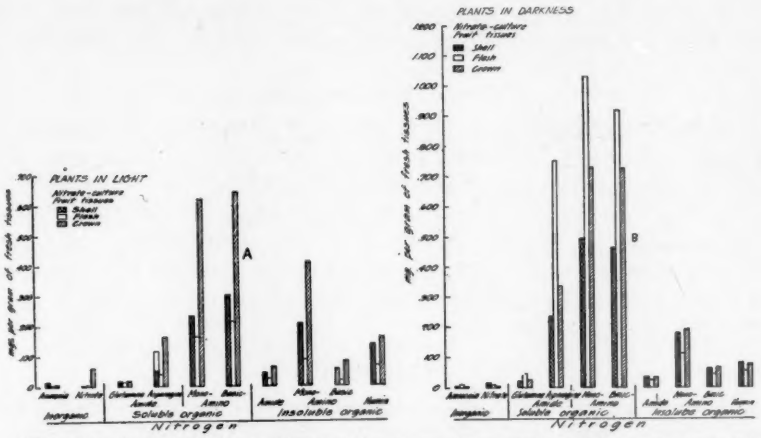

FIG. 11. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the flesh, shell and crown of the fruit of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the flesh, shell and crown of the fruit of pineapple sister shoots grown in solution cultures and kept in darkness for 4 months.

plants kept in darkness. The quantities of asparagine were exceedingly small in the flesh section of fruits of the plants kept in light but in appreciably greater quantities in the shell and crown sections of the fruit from the same group of plants.

3. The quantities of soluble mono-amino and basic nitrogen were considerably greater in the sections of the fruit from plants kept in darkness than those kept in light.

4. Insoluble organic or protein nitrogen was, with a few exceptions, present in greater quantities in the fruits of plants kept in light than those kept in darkness.

The data for the distribution of nitrogen in the roots of the plants kept in light, as presented in table II, show the following:

1. Ammonium nitrogen was present only in traces while nitrate was in comparatively large amounts. The relatively high concentrations of nitrate and small amounts of ammonium in pineapple roots have been amply discussed in former studies (7).

2. With respect to the distribution of the various fractions of organic nitrogen, only the amounts of humin nitrogen were larger in the present than in our former studies, a condition which may be attributed either to a more advanced chronological age and physiological maturity of the tissues or to some unsuspected modification of our technique.

No comparable data were available for plants of the series kept in darkness because the roots were weak and by the time of harvest many had died.

Distribution of sugars

Table IV and figures 12-A and 12-B presenting the distribution of sucrose, reducing, and total sugars in the various sections of the leaves, stem, and fruit of the plants of both series show the following:

1. The amounts of reducing and of total sugars in the different leaf sections of both lots of plants showed no outstanding differences. In certain cases the leaf sections of the plants kept in darkness contained larger amounts of both reducing and total sugars than those of the plants grown in light. The cause for this is unknown.

TABLE IV

PERCENTAGE REDUCING, TOTAL SUGARS, AND SUCROSE, ON A FRESH WEIGHT BASIS, IN DIFFERENT SECTIONS OF PINEAPPLE LEAVES, STEM, AND FRUIT OF TWO SETS OF PINEAPPLE PLANTS, ONE EXPOSED TO LIGHT AND THE OTHER KEPT IN DARKNESS SINCE AN EARLY STAGE OF FRUIT DEVELOPMENT

Plant and fruit sections	In light			In darkness		
	Total	Reducing	Sucrose	Total	Reducing	Sucrose
Leaves	%	%	%	%	%	%
Mature						
C1 + D1 (Base)	2.27	1.74	0.53	2.52	2.33	0.19
C2, 3 + D2, 3	2.40	1.74	0.66	2.56	2.29	0.27
C4 + D4	2.91	1.88	1.03	.....	.....	.....
C5 + D5 (Tip)	3.09	1.97	1.12	.....	.....	.....
Active						
E1 + F1 (Base)	2.33	1.82	0.51	1.93	1.72	0.21
E2 + F2	2.36	1.95	0.41	2.58	2.38	0.20
E3 + F3	1.77	1.01	0.76	2.58	2.35	0.23
E4 + F4	2.07	1.03	1.04	.....	.....	.....
E5 + F5 (Tip)	2.59	1.28	1.31	.....	.....	.....
Stem						
A + C (Lower)	1.77	1.19	0.58	1.16	0.92	0.24
D + F (Upper)	2.19	1.54	0.65	0.75	0.45	0.30
Peduncle	3.30	1.46	1.84	1.24	1.12	0.12
Fruit						
Shell	6.08	2.25	3.83	1.34	1.14	0.20
Flesh	9.66	2.80	6.88	1.47	1.40	0.07
Crown	2.16	1.52	0.64	0.67	0.67	0.00
Roots	0.44	0.23	0.21	0.05	0.05	0.00

2. Sucrose, although usually found in relatively small amounts in the leaves, was present, comparatively, in considerably greater quantities in the leaves of the plants kept in light than in the corresponding sections of those of the plants kept in darkness.

3. The amounts of both reducing sugars and sucrose were consistently larger in the stem of the plants kept in light than in those kept in darkness.

The difference between the quantities of sugars in tissues of the two lots of plants was greater for the younger, upper, tissues of the stem (D–E section) and for the peduncle, than for the more aged tissues of the lower (A–C) stem section. Sucrose, being possibly a storage product (**6**), was practically absent in the peduncle and in the fruit tissues of the plants kept in darkness, but was very abundant in the same tissues of the plants kept in light.

The presence of relatively large amounts of reducing sugars in the leaves of the plants kept in darkness cannot be explained satisfactorily.

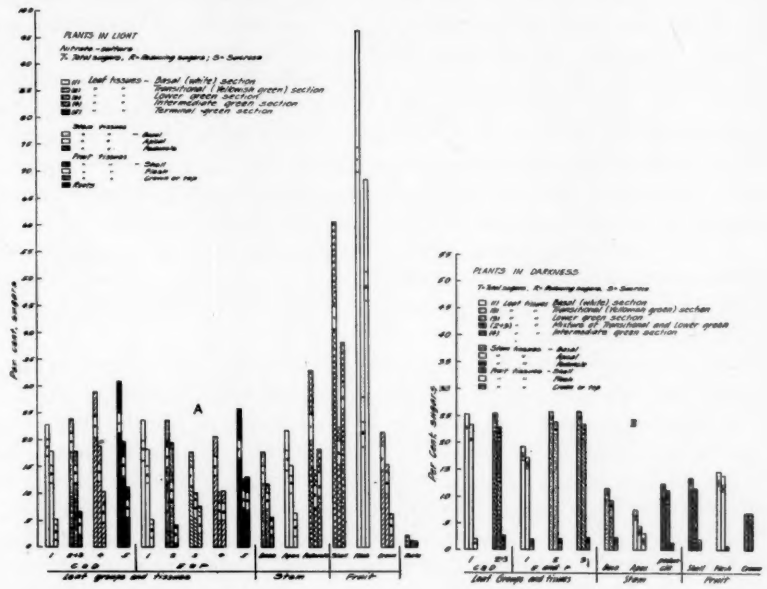

FIG. 12. A, distribution of reducing, total sugars and sucrose in different sections of leaf groups C, D, E, and F, and of the stem, fruit, and roots of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of reducing, total sugars, and sucrose in different sections of leaf groups C, D, E, and F, and of the stem, fruit, and roots of pineapple sister shoots grown in solution cultures and kept in darkness for 4 months.

Discussion

The foregoing data show that the chemical composition of the tissues of pineapple plants and fruits kept in darkness differs considerably from that of plants kept in light.

Pineapple fruits grown in darkness contain very small amounts of sugars and organic acids when contrasted with similar fruits grown in light. The acidity of the flesh tissues of fruits grown either in light or in darkness

did not differ as much as that of the shell tissues. The greater acidity of the shell tissues of the fruits grown in light was probably due to the presence of greater amounts of carbohydrates, probably synthesized by the chlorophyllous tissues of the shell. It is also possible that the greater differences in acidity of the shell than of the flesh tissues in the two sets of fruits were due to the translocation of smaller amounts of sugars to the tissues of the shell than to those of the flesh of the fruits kept in darkness. Considering the fact that the chlorophyllous tissues of the shell of the fruits grown in darkness were unable to synthesize carbohydrates in darkness, and also that they are located at a greater distance from the core (the main artery for sugar supply) than the flesh, the greater difference in the acidity of shell than of flesh tissues as mentioned above, can be explained. Certain unpublished data by the authors have shown that extremely low carbohydrate supplies are often associated with the production of very small amounts of organic acids, in both the leaves and fruits of pineapple plants. In contrast with the above statement, it was observed in certain other unpublished experiments, that the amounts of organic acids in the fruit tissues of plants placed in darkness increased to very high levels if the fruits had approximately completed growth, but had not yet ripened at the time the plants were subjected to darkness. In this particular experiment the carbohydrate content of the fruits was relatively high.

With respect to assimilation and distribution of nitrogen, the data show that pineapple plants kept in darkness for a period of four months accumulated in the upper section of the stem, in the peduncle and in the flesh of the fruit, great quantities of soluble organic nitrogen consisting of asparagine and of various amino acids; the latter are reported in this paper collectively as mono-amino and basic nitrogen. Protein was present only in slightly greater amounts in the stem of the plants exposed to light than in those kept in darkness. Nearly all fractions of protein were from two to four times greater in the leaves of the plants exposed to light than in those kept in darkness.

The data show, in general, that there was a tendency for soluble organic nitrogen to accumulate in greater quantities in the non-chlorophyllous, and particularly in the meristematic regions, than in the highly differentiated chlorophyllous tissues of the plants kept in darkness. In the chlorophyllous sections of the leaves and in the lower section of the stem (composed mostly of thoroughly developed and mature tissues) the accumulation of soluble organic nitrogen was not as great as in the upper stem sections.

In the non-chlorophyllous sections of the leaves and fruit and in the upper section of the stem of the plants kept in darkness, the quantity of protein decreased either because its synthesis was inhibited through the lack of sufficient carbohydrates, or its disappearance was favored by enzymic hydrolysis.

In the chlorophyllous and in the more mature tissues of the same organs, protein was present in greater quantities either because its disappearance through enzymic hydrolysis was partially inhibited, or its synthesis was favored, to a certain extent, by the presence of carbohydrates.

Tables V and VI and figures 13 and 14, presenting the distribution of

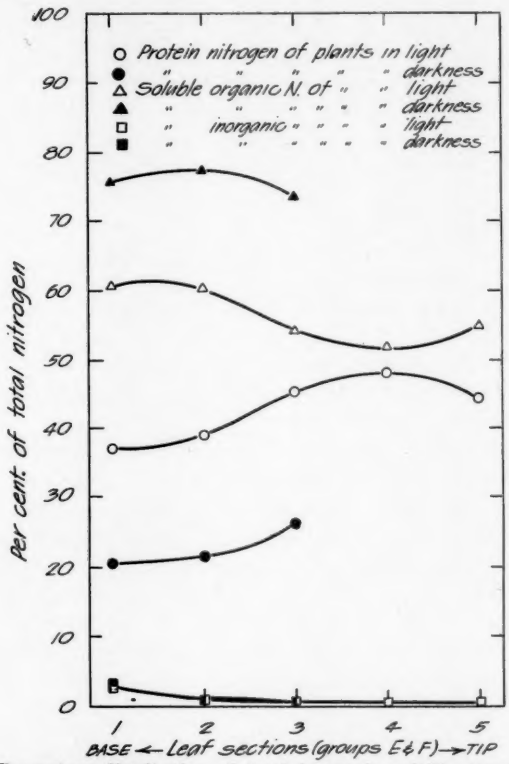

FIG. 13. Percentage distribution of total inorganic, soluble organic, and protein nitrogen in different sections of the E and F groups of leaves of pineapple sister shoots grown in nutrient solutions and exposed either to light or kept in darkness.

inorganic, soluble organic, and protein (insoluble organic nitrogen) on a percentage basis, show some very interesting relationships between the various nitrogen fractions in the two different lots of plants.

In sections 1 and 2, *i.e.*, the basal sections, of the mature C and D leaf groups, quantities of soluble organic nitrogen were 1.285, and protein 1.605 times, greater in the plants exposed to the light in the greenhouse than in those kept in the darkened box.

In sections 1, 2, and 3 of the younger and active E and F leaf groups, quantities of soluble organic nitrogen were 1.300, and protein 0.559 times, greater in the plants kept in darkness than in those exposed to light. If the same data are compared, however, on a percentage basis, as in figure 13, and not on a basis of actual amounts of nitrogen, slightly different results are

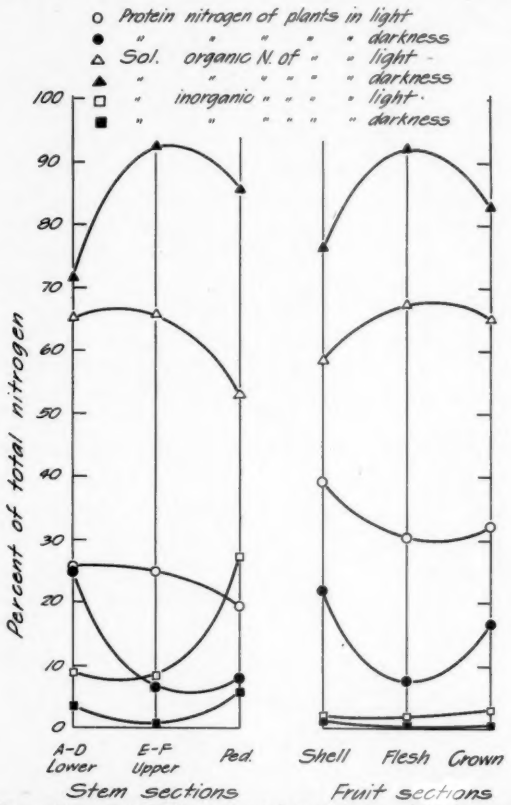

FIG. 14. Percentage distribution of total inorganic, soluble organic, and protein nitrogen in different sections of the stem and in the flesh, shell, and crown of the fruit of pineapple sister shoots grown in nutrient solutions and either exposed to light or kept in darkness.

obtained because of the difference in the amounts of nitrogen absorbed by the two lots of plants. The values for soluble organic, but not for protein nitrogen, were higher for the sections of the younger E and F leaf groups than for the more mature C and D groups of the plants kept in darkness, whereas the opposite condition prevailed in those exposed to light. The data,

TABLE V

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC, AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT TISSUES OF PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES CONTAINING NITRATE AND EXPOSED TO SEASONAL LIGHT UNDER GREENHOUSE CONDITIONS FOR FIVE MONTHS, TOGETHER WITH PERCENTAGE VALUES FOR THE SAME AND FOR THEIR FRACTIONS

Plant sections	Inorganic nitrogen				Soluble organic nitrogen						Insoluble organic nitrogen						Total N
	Ammonium	Nitrate	Total	Total	Glutamine	Asparagine	Monoamino	Basic	Total	Total	Amide	Monoamino	Basic	Humin	Total	Total	
Leaves	%	%	*mg.*	%	%	%	%	%	*mg.*	%	%	%	%	%	*mg.*	%	*mg.*
Mature																	
C1 + D1 (Base)	23.0	77.0	0.002	1.8	0.9	11.2	47.2	40.7	0.904	63.6	14.1	43.5	15.9	26.5	0.490	34.6	1.416
C2 + D2	44.5	55.5	0.011	0.7	1.2	9.7	43.1	46.0	0.921	58.2	8.7	54.0	10.0	27.3	0.649	41.1	1.581
C4 + D4	43.5	56.5	0.012	0.4	1.1	11.2	40.9	46.8	1.482	53.9	6.9	59.6	12.8	20.7	1.256	45.7	2.750
C5 + D5 (Tip)	7.5	92.5	0.019	0.2	1.0	12.9	38.6	47.5	1.653	52.8	8.9	54.0	10.9	26.4	1.463	47.0	3.135
Active																	
E1 + F1 (Base)	16.4	83.6	0.045	2.7	1.1	12.1	42.6	44.2	0.971	60.6	16.6	46.3	9.0	28.1	0.588	36.7	1.604
E2 + F2	28.5	71.5	0.008	0.7	0.9	13.5	40.9	44.7	0.823	60.3	7.4	57.8	8.8	26.0	0.534	39.0	1.365
E3 + F3	70.1	29.9	0.011	0.7	0.8	5.6	45.8	47.8	1.268	53.8	5.5	60.5	12.7	21.3	1.075	45.5	2.357
E4 + F4	52.7	47.3	0.015	0.5	1.4	15.3	37.1	46.2	1.499	51.8	5.0	59.2	14.2	21.6	1.380	47.7	2.895
E5 + F5 (Tip)	63.9	36.1	0.022	0.5	1.2	5.5	44.3	49.0	1.816	55.0	5.9	60.1	12.9	21.1	1.471	44.5	3.308
Stem																	
A + D (Lower)	1.8	98.2	0.201	8.9	1.4	4.8	46.3	47.5	1.467	65.3	5.9	52.5	14.5	27.1	0.580	25.8	2.249
E + F (Upper)	8.0	92.0	0.163	8.6	2.3	9.8	40.8	47.1	1.189	66.3	6.9	63.5	8.9	20.7	0.474	25.1	1.885
Peduncle	0.8	99.2	0.360	27.8	0.9	6.1	45.2	47.8	0.682	52.8	17.6	43.5	12.6	26.3	0.251	19.4	1.292
Fruit																	
Shell	84.3	15.7	0.020	1.8	2.5	17.2	34.8	45.5	0.662	58.8	10.0	46.5	12.7	30.8	0.443	39.4	1.126
Flesh	56.2	43.8	0.011	1.8	3.1	9.1	38.4	49.4	0.431	67.4	13.9	43.3	9.1	33.7	0.197	30.8	0.640
Crown	12.5	87.5	0.069	3.0	1.3	11.2	42.7	44.8	1.443	65.0	9.0	57.8	11.2	22.0	0.712	32.0	2.223
Roots	6.7	93.3	0.112	6.7	3.1	11.9	45.3	39.7	0.901	53.4	8.3	48.6	11.5	31.6	0.672	39.9	1.684

TABLE VI

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC, AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT TISSUES OF PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES CONTAINING NITRATE AND KEPT IN DARKNESS FOR FOUR MONTHS, TOGETHER WITH PERCENTAGE VALUES FOR THE SAME AND FOR THEIR FRACTIONS

Plant sections	Inorganic nitrogen				Soluble organic nitrogen						Insoluble organic nitrogen						Total N
	Ammonium	Nitrate	Total	Total	Glutamine	Asparagine	Monoamino	Basic	Total	Total	Amide	Monoamino	Basic	Humin	Total	Total	
Leaves	%	%	*mg.*	%	%	%	%	%	*mg.*	%	%	%	%	%	*mg.*	%	*mg.*
Mature																	
C1 + D1 (Base)	5.0	95.0	0.034	2.9	0.9	9.9	40.9	48.3	0.812	69.5	8.0	50.15	19.15	22.7	0.323	27.6	1.169
C2 + D2	4.2	95.8	0.046	4.3	1.1	14.9	39.3	45.2	0.608	58.5	10.0	46.3	14.5	29.2	0.387	37.2	1.040
Active																	
E1 + F1 (Base)	6.4	93.6	0.047	3.3	1.0	20.0	39.7	39.3	1.066	76.0	7.0	51.8	19.3	22.0	0.290	20.7	1.403
E2 + F1	16.0	84.0	0.017	0.9	1.1	12.5	42.9	43.5	1.332	77.3	5.6	51.1	17.3	25.0	0.376	21.8	1.725
E3 + F3 (Green)	39.0	61.0	0.008	0.2	0.5	19.5	38.9	41.1	1.577	73.3	6.6	47.8	16.4	29.2	0.563	26.3	2.148
Stem																	
A + D (Lower)	7.2	92.8	0.079	3.4	0.8	19.4	40.2	39.6	1.628	71.6	7.6	49.8	15.7	26.9	0.568	25.0	2.275
E + F (Upper)	35.5	64.5	0.049	0.8	1.0	18.8	45.0	35.2	5.254	92.4	6.7	57.0	19.0	17.3	0.384	6.8	5.686
Peduncle	5.5	94.5	0.163	6.1	1.7	30.5	37.6	30.2	2.256	86.2	10.7	42.5	19.8	27.0	0.198	7.7	2.616
Fruit																	
Shell	29.0	71.0	0.020	1.3	1.8	19.3	40.6	38.3	1.215	76.5	9.9	50.8	16.6	22.7	0.353	22.2	1.588
Flesh	58.0	42.0	0.019	0.3	1.7	27.2	37.6	33.5	2.744	91.8	11.1	47.7	17.8	23.4	0.235	7.9	2.997
Crown	38.8	61.2	0.010	0.4	1.4	18.4	40.2	40.0	1.815	83.0	9.0	53.3	17.0	20.7	0.363	16.6	2.188

if our interpretation is correct, indicate that, in the absence of light, protein synthesis and accumulation were interrupted to a greater degree in the sections of the younger E and F leaf groups than in those of the older C and D leaf groups.

The percentage distribution of soluble organic and protein nitrogen in the stem sections of the plants of the two different treatments presents very sharp contrasts. The amounts of soluble organic nitrogen in the mature lower A–D sections of the stem of the plants kept in darkness were 1.110 times greater than those in light. There was hardly any difference between the percentage amounts of protein nitrogen in the same stem sections of the two lots of plants.

In the younger tissues of the upper D–E section of the stem the percentage of soluble organic nitrogen was 66.3 for the plants exposed to light and 92.4 for those kept in darkness. The percentage value for protein nitrogen for the former plants was 25.1 and for the latter group 6.8. If we consider, however, the actual amounts of soluble organic nitrogen in the upper D–E stem section, we find 1.189 mg. per gram of fresh weight for the plants exposed to light and 5.254 mg. per gram of fresh weight for those kept in darkness, or 4.42 times larger amounts for the latter lot. The actual amounts of protein nitrogen in the upper D–F stem section were 0.474 mg. per gram of fresh tissues for the plants exposed to light and 0.384 mg. per gram of fresh tissues in corresponding sections of plants kept in darkness, or only 1.235 times greater quantities in favor of the former lot. All such comparisons show that protein did not accumulate in the tissues in amounts equal to those of soluble organic nitrogen. The relatively small difference of 0.090 mg. of nitrogen between the amounts of protein nitrogen in the plants exposed to light and in those kept in darkness suggests that very small amounts of protein underwent enzymic hydrolysis and thereby increased the quantities of soluble organic nitrogen. Consequently, the accumulations of soluble organic nitrogen may have resulted from directly assimilated inorganic (nitrate) nitrogen without further conversion to protein, and not from hydrolyzed proteins. The data suggest that the processes for the conversion of soluble organic to protein nitrogen were retarded in the young, or upper D–F, stem section of the plants kept in darkness because these tissues lacked sufficient amounts of available carbohydrates and possibly other substances essential for protein synthesis. As is shown in table I and figure 2, the cessation of additional growth may have resulted in the accumulation of great quantities of soluble organic nitrogen. This was possibly caused by the inability of the tissues to synthesize protein in the absence of carbohydrates, etc., in the plants kept in darkness.

We do not wish, however, to give the impression in the above statement that protein hydrolysis by proteoclastic cellular enzymes is not a

regular feature of nitrogen catabolic processes. Our interpretation of the data is that, under the nutritional conditions of our experiments, where an ample external supply of inorganic nitrogen was available, the plants absorbed some of this supply and assimilated it only to the stage of soluble organic nitrogen and that, in the presence of great amounts of soluble organic nitrogen, protein hydrolysis was partly inhibited. This fact is well brought out by the examination of the values for the actual amounts and percentage values of protein nitrogen in the tissues of the mature lower (A–C) stem section. These values are approximately the same for the plants of both series, indicating that the effects of enzymic hydrolysis on protein catabolism were insignificant.

The amounts of soluble organic and protein nitrogen in the chlorophyllous sections of the leaves of groups C–D, which were dead after the plants had been kept for four months in continuous darkness, are presented in table VII. The values presented in this table, when contrasted with those for total soluble and insoluble organic nitrogen of comparable sections, as presented in table V, show that approximately two-thirds

TABLE VII

MILLIGRAMS OF SOLUBLE ORGANIC AND PROTEIN NITROGEN PER GRAM OF FRESH TISSUE IN THE DEAD CHLOROPHYLLOUS SECTIONS 4 AND 5, OF LEAF GROUPS C–D AND E–F OF PLANTS KEPT IN DARKNESS FOR FOUR MONTHS

PLANT SECTIONS	NITROGEN			
	SOLUBLE ORGANIC	PROTEIN	TOTAL	RATIO OF PROTEIN TO SOLUBLE ORGANIC
	mg.	*mg.*	*mg.*	
Leaves				
$C–D_{4+5}$	0.480	0.785	1.285	1.64
$E–F_{4+5}$	0.430	0.745	1.175	1.73

of the total soluble organic and one-half of the protein nitrogen migrated from the dead sections of the leaves to the stem. This assumption is made on the supposition that large quantities of soluble organic and protein nitrogen existed in the chlorophyllous sections of the leaves of the plants kept in the dark. Theoretically, the formation of great amounts of protein in the leaves of the plants kept in darkness is incompatible with the facts. We may assume, however, that organic nitrogen was present in both the plants kept in light and in darkness in approximately equal quantities and that the amounts of nitrogen represented by the difference between soluble organic and protein nitrogen in sections 4 and 5 of leaf groups C–D and E–F of the plants kept in light, as shown in table V, and that of the plants kept in darkness, as shown in table VII, all migrated into the tissues of the stem

of the plants kept in darkness. The amounts of migrated nitrogen represented by this difference are as follow: soluble organic: 1.155 (1.610 – 0.455); protein: 0.625 (1.390 – 0.765); total: 1.780. Thus the value for the amount of soluble organic nitrogen which migrated from the dead chlorophyllous sections of the leaves to the stem has been set at 1.155 mg. and for protein at 0.625 mg. of nitrogen per gram of fresh tissues. The total fresh weight of the dead sections was approximately 100 gm. and that of the stem, 120 gm. The influx of nitrogen from the leaves to the stem would have increased the original concentration, on the basis of these calculations, by 1.48 mg. per gram of fresh tissue. The average total soluble nitrogen concentration per gram of fresh tissue in the combined two sections of the stem and peduncle was 1.10 mg. for the plants growing in light and 3.04 mg. for the plants growing in the dark. If we subtract 1.48 mg. from 3.04 mg. we obtain 1.56 mg. which represents the concentration of soluble organic nitrogen per gram of fresh tissue in the combined sections of the stem and peduncle of the plants kept in darkness, after substracting the amounts of nitrogen which moved to the stem and peduncle from the dead sections of the leaves. If all these assumptions and calculations are correct it is then safe to state that a great portion of the soluble organic nitrogen of the stem and peduncle of the plants kept in darkness was derived from the nitrogen reserves in the dead sections of the leaves.

The data for the chlorophyllous sections of the leaves and that for the upper stem indicate, to some extent, that protein hydrolysis had operated under the experimental conditions in the darkened box, liberating both carbohydrates and soluble nitrogen. It seems possible that the liberated carbohydrates were used to supply the energy requirements of the protoplasm and that soluble organic nitrogen accumulated. This view is in harmony with that of NIGHTINGALE (5) who suggests that the proteins of plants subjected to prolonged darkness are respired as a source of energy.

The percentage distribution of soluble organic and protein nitrogen in the peduncle of the plants of the two series followed, in spite of differences in the actual amounts, approximately the same trend as in the upper D–E stem sections.

The percentage distribution of different nitrogenous fractions in the tissues of the flesh, shell, and crown of the fruit varied considerably with the great diversity in function of these organs. The flesh, consisting entirely of non-chlorophyllous tissues, serves as a storage organ. The sepals and bracts of the shell, containing fair quantities of chlorophyll, act as photosynthetically assimilating organs until the time of the ripening of the fruit; while the petals, stamens, and style undergo all the morphological and metabolic processes which accompany flowering, growth, and ripening of the fruit. The crown may be compared morphologically and physiologically

to a small pineapple plant attached to a living substratum, but with its leaves and stem functioning autotrophically as plants grown in organic substrata.

The flesh of the fruit, being a storage organ, compares, in the order of the distribution of the fractions of soluble organic and protein nitrogen, more nearly with the stem and peduncle than with the leaves. Likewise, the shell compares, in this respect, more nearly with the leaves than with the stem or peduncle.

An examination of the actual amounts of insoluble nitrogen in the flesh of the fruit from the plants exposed to light shows that there were 0.197 mg. per gram of fresh tissues, while in the flesh of the fruits from plants kept in darkness the amount was 0.235 mg. per gram of fresh tissues or a difference of 0.038 mg. in favor of the latter series. This difference in favor of the plants in darkness is contrary to all expectations. It may be explained, however, by the fact that the weights of the fruits from the latter plants, as well as the sugars and organic acids content of their tissues, were considerably less than in the fruits of the plants grown in light. Consequently, the concentrations of nitrogen per volume of tissues were not distributed through as great a volume of tissues in the fruits from the plants in darkness as they were in those from the plants in light.

Table VIII, reporting the ratio values of soluble organic to protein

TABLE VIII

RATIO OF SOLUBLE ORGANIC TO INSOLUBLE ORGANIC NITROGEN IN DIFFERENT LEAF, STEM, AND FRUIT SECTIONS OF PINEAPPLE PLANTS GROWN EITHER IN THE SEASONAL LIGHT OF THE GREENHOUSE FOR FIVE MONTHS OR KEPT IN A DARK BOX FOR FOUR MONTHS

PLANT SECTIONS	IN DARKNESS	IN LIGHT
	SOLUBLE ORGANIC-N / PROTEIN-N	SOLUBLE ORGANIC-N / PROTEIN-N
Leaves		
Mature		
C1 + D1 (Base)	2.515	1.845
C2 + D2	1.572	1.418
Active		
E1 + F1 (Base)	3.680	1.652
E2 + F2	3.540	1.540
E3 + F3 (Green)	2.800	1.180
Stem		
A + D (Lower)	2.868	2.530
E + F (Upper)	13.670	2.510
Peduncle	11.400	2.320
Fruit		
Shell	3.400	1.495
Flesh	11.680	2.190
Crown	5.000	2.025

nitrogen, shows that, in the various sections of the plants exposed to light, the values for soluble organic nitrogen were from 1.18 to 2.53 times in excess of those for protein; whereas in plants kept in darkness, the former were from 1.572 to 13.670 times in excess of the latter. The ratio values of soluble organic to protein nitrogen were generally higher for the non-chlorophyllous and lower for the chlorophyllous sections of the plant and fruits.

In general, the amounts of soluble mono-amino and basic nitrogen fractions of the plants exposed to light were especially large when compared with those which were obtained in our former studies (7) with nitrate nutrition. On the basis of a similar comparison the mono-amino and basic fractions of insoluble organic or protein nitrogen were low. Total organic nitrogen values, however, were lower in the present than in our former studies (7). The low values obtained were possibly a result of the inhibiting effects of darkness on the synthesis of carbohydrates and other substances essential for protein production. It is also possible that a decreased rate of nitrogen absorption from the nutrient solution, as the result of the weakened condition of root systems of the plants of this series, may have been the cause for the small amounts of protein nitrogen found in the tissues. The greater quantities of soluble, and less of insoluble, nitrogen fractions in the plants of the present study in comparison to those of our former studies are probably attributable to differences in the chronological and physiological age of the two groups of plants. Amounts of humin nitrogen were comparatively large, being approximately twice as large as they were in the former studies (7). Although it is possible that the relatively large amounts of humin nitrogen are a result of the hydrolysis in the nitrogen fraction determinations, it is improbable that such a condition could have developed, because the same technique was employed in all cases. Humin nitrogen was found to increase with tissue age and maturity. It is, therefore, plausible to assume that the production of relatively great quantities of humin nitrogen was a result of the comparatively high rate of tissue maturation of leaf groups E and F during fruit development.

There was no outstanding difference in the distribution of either reducing or total sugars in the various sections of the plants of both series. Sucrose, however, although present in small quantities, was found in consistently greater quantities in the plants exposed to light than in those kept in darkness. This fact is brought out very well in table IX where the sucrose contents of the plants of the two series are contrasted on a ratio basis. The values of sucrose in the leaves were from 1.37 to 3.30 times greater for plants grown in light than for those grown in darkness. The peduncles of the plants grown in light contained 15.35 times more sucrose than the corresponding organs of the plants grown in darkness. The shell and flesh of the fruits of the plants kept in light contained 19.30 and 98.50 times, respectively, more sucrose than those kept in darkness.

TABLE IX

RATIO OF SUCROSE CONTENT OF DIFFERENT LEAF, STEM, AND FRUIT SECTIONS OF PLANTS KEPT IN THE SEASONAL LIGHT OF THE GREENHOUSE FOR FIVE MONTHS TO THAT OF THE SAME SECTIONS OF PLANTS KEPT IN DARKNESS FOR FOUR MONTHS

PLANT SECTIONS	$\frac{\text{S.P.L.*}}{\text{S.P.D.}}$
Leaves	
Mature	
C1 + D1 (Base)	2.79
C2 + D2	1.37
Young active	
E1 + F1 (Base)	2.43
E2 + F2	2.05
E3 + F3	3.30
Stem	
A + C (Lower)	2.42
D + E (Upper)	2.08
Peduncle	15.35
Fruit	
Shell	19.30
Flesh	98.50
Crown	.....

* S.P.L. = Sucrose in plants kept in light.
S.P.D. = Sucrose in plants kept in darkness.

The data as presented above suggest that the relative quantities of sucrose, more so than those of reducing sugars, in the tissues of the stem and fruit, or of any other such storage organs, may serve as indices for the relative activity of the photosynthetic mechanism of plants under different light conditions. It is unfortunate that analytical data for starches, hemicellulose, and other carbohydrates are lacking. The data, however, regarding the distribution of sucrose in fruit and stem under the conditions of this experiment support the often made allegation that sucrose is a storage sugar. The small amounts of sucrose in the leaves of the plants kept in darkness may indicate either its utilization for synthetic or respiratory processes without subsequent replenishment of this substance, or hydrolysis and possible translocation to other parts of the plant.

The results support our view, in general, that light in relatively sufficient amounts favored protein and sucrose accumulation and darkness favored accumulations of soluble organic nitrogen.

A few comparisons between our data and those of VICKERY *et al* (9) show many similarities with respect to the distribution of organic solids, total organic acidity, etc., between pineapple and tobacco plants under similar light conditions.

Summary

Two pairs of pineapple shoots obtained from two different mother plants were employed for these studies. The two members of each pair were of

approximately the same weight and degree of development. They were detached from their mother plants in February 1935 while at the red bud stage. After weighing they were placed in solution cultures in the greenhouse and allowed to produce roots. When all had developed vigorous and extensive root systems one of the two members from each pair of shoots was transferred to a dark box where it remained for the subsequent four months, while the other one was left in the greenhouse for five months. The fruits of the shoots kept in the darkened box ripened one month earlier than those in the greenhouse. At harvest, the leaves, stem and fruit were sectioned in order to separate senile from mature and the latter from young tissues and also chlorophyllous from non-chlorophyllous tissues. The results obtained were as follows:

1. The total weight of the plants, namely, the plant plus the fruit, growing in light increased 89 per cent. during the period of five months, while that of plants growing in darkness decreased 12.8 per cent. The increase in the former case was entirely due to the growth of the fruit, and the loss in the latter to the drying of the terminal section of the leaves. The weight of the fruit plus that of the crown was 3.28 times greater for the plants growing in light than for those growing in darkness.

2. The chemical properties of the fruits of the two series differed. The values for titratable acidity, reported as citric acid, of the shell was 3.27 times greater for the fruits from plants in light than for those in darkness.

3. The amounts of total soluble solids in the shell, determined refractometrically, were 3.15 times larger for the fruits from the plants in light than for the fruit from those in darkness. In the flesh they were 2.98 times larger for the former than for the latter series.

4. The distribution of different fractions of inorganic, soluble organic, and protein nitrogen differed considerably in different sections of the leaves, in the stem, and in the fruits of the plants exposed to light and those kept in darkness. Ammonium was present only in traces in nearly all plant sections. Nitrate was found in relatively large amounts in the peduncle, stem, and in the non-chlorophyllous basal sections of the leaves, in all other sections it was found in small amounts. Soluble organic nitrogen (consisting mostly of amino-, basic- and asparagine-nitrogen) was abundant in the meristematic and in the non-chlorophyllous sections of the leaves, while protein was most abundant in the chlorophyllous and highly differentiated tissues of the various organs. The ratio values of soluble organic to protein nitrogen were considerably greater for the leaf and stem tissues and fruits of the plants grown in darkness than for those of the plants grown in light, indicating that in the former series the assimilation of inorganic nitrogen had proceeded only to the stage of soluble organic but not to that of protein nitrogen.

5. No appreciable differences could be observed in the amounts of reduc-

ing sugars in the tissues of the two lots of plants. Sucrose, however, although present in small quantities in all vegetative organs, was consistently greater in the plants kept in light than in those kept in darkness. The leaves and stem of the plants exposed to light had from 1.4 to 3.3 times more sucrose than the same organs of the plants grown in darkness. The peduncle, shell, and flesh of the fruits of the plants in light contained 15, 19 and 99 times, respectively more sucrose than the corresponding sections of the fruits of the plants in darkness. The results have indicated rather conclusively that the accumulation of sucrose in storage organs under the minus-light conditions of these experiments was practically nil.

PINEAPPLE EXPERIMENT STATION
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CHANGES IN THE COMPOSITION AND RATE OF GROWTH ALONG THE DEVELOPING STEM OF ASPARAGUS

C. W. CULPEPPER AND H. H. MOON

(WITH SIX FIGURES)

Introduction

In view of the wide use of the tender immature stem of asparagus (*Asparagus officinalis* L.) as a food product and the rapid changes that occur in its composition at the stages of development desirable for table use, it appears advantageous to have a rather detailed history of the physical and chemical changes that take place during the entire period of its growth. A considerable amount of information concerning its growth and composition has been obtained but the story is complicated by many factors. The growth of the plant has been studied by BÜCHNER (**3**), WICHERS and TOLLENS (**13**), and WORKING (**14**). The composition of the stalk has been studied by BISSON, JONES, and ROBBINS (**2**), CULPEPPER and MOON (**5**), NIGHTINGALE and SCHERMERHORN (**10**), and MORSE (**8, 9**). The purposes of these several investigations differed widely.

No very thorough study of the composition of the developing stem in different zones as related to the rate of growth in these zones has been made. The purpose of this report is to present the results of some investigations bearing on this relationship.

Materials and methods

The material employed in these studies was grown in the field at the Arlington Experiment Farm, Rosslyn, Va. Details of its culture have been given in an earlier report (**6**) upon the effect of temperature upon the elongation of the growing stem. Material for both studies was grown in the same plot with identical cultural treatment.

The rate of growth was determined by direct measurement of the increase in the length of short segments, marked out by suitable methods previously described (**6**). The measurements thus made have been recorded as the increase in length in centimeters of segments one cm. long during a time interval of 24 hours. The values are therefore read in cm. per cm. per 24 hours and are averages of 10 to 64 readings obtained as described in the earlier report (**6**).

The readings used in these studies were limited to those taken between 65° and 70° F., the average being close to 67.5° F. This, however, included readings made for many different zones along stalks of 14 different heights. The method of obtaining and recording the data will be clear from table I.

Each series of samples for chemical analysis was taken from 20 to 30

TABLE I

THE RATE OF GROWTH OF STALKS OF ASPARAGUS OF DIFFERENT HEIGHTS IN VARIOUS REGIONS IN THE GROWING ZONE. EXPRESSED IN CM. OF GROWTH PER CM. PER 24 HOURS

Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth
Total height 15 cm.		Total height 45 cm.		Total height 75 cm.		Total height 105 cm.		Total height 135 cm.		Total height 165 cm.	
cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
3.2	0.000	11.7	0.000	33.4	0.000	68.0	0.000	103.0	0.000	140.6	0.000
5.0	0.170	16.0	0.100	40.0	0.117	75.0	0.100	108.0	0.056	144.0	0.060
7.0	0.465	20.0	0.222	45.0	0.206	80.0	0.207	112.0	0.137	148.0	0.165
8.0	0.575	24.0	0.370	50.0	0.309	85.0	0.365	116.0	0.235	152.0	0.330
9.0	0.658	28.0	0.536	55.0	0.477	88.0	0.477	120.0	0.406	154.0	0.438
.....	.....	32.0	0.650	60.0	0.624	92.0	0.600	124.0	0.580	156.0	0.560
10.0	0.701	34.9	0.682	64.8	0.664	95.5	0.647	126.4	0.628	157.6	0.609
11.0	0.674	37.0	0.635	67.0	0.615	97.0	0.620	128.0	0.605	159.0	0.595
12.0	0.570	39.0	0.557	69.0	0.552	99.0	0.568	129.0	0.585	161.0	0.522
13.0	0.440	41.0	0.458	71.0	0.463	101.0	0.485	131.0	0.510	163.0	0.400
.....	.....	43.0	0.317	73.0	0.338	103.0	0.330	133.0	0.380	.....	.....
15.0	0.000	45.0	0.000	75.0	0.000	105.0	0.000	135.0	0.000	165.0	0.000
Total height 25 cm.		Total height 55 cm.		Total height 85 cm.		Total height 115 cm.		Total height 145 cm.		Total height 175 cm.	
5.5	0.000	16.6	0.000	44.0	0.000	79.6	0.000	115.0	0.000	153.1	0.000
7.0	0.087	23.0	0.117	50.0	0.076	85.0	0.078	120.0	0.060	157.0	0.080
9.0	0.200	28.0	0.242	55.0	0.170	90.0	0.167	125.0	0.170	160.0	0.180
11.0	0.350	33.0	0.395	60.0	0.291	95.0	0.297	130.0	0.354	163.0	0.315
13.0	0.500	38.0	0.565	65.0	0.450	98.0	0.420	135.0	0.577	166.0	0.460
15.0	0.645	42.0	0.654	70.0	0.597	102.0	0.587	136.8	0.622	167.0	0.573
17.0	0.695	44.4	0.677	75.0	0.658	105.7	0.640	138.0	0.603	168.0	0.603
19.0	0.661	47.0	0.629	77.0	0.612	107.0	0.612	139.0	0.580	169.0	0.594
20.0	0.610	49.0	0.560	79.0	0.557	109.0	0.557	141.0	0.510	171.0	0.525
21.0	0.545	51.0	0.465	81.0	0.475	111.0	0.485	143.0	0.380	173.0	0.395
22.0	0.463	53.0	0.320	83.0	0.342	113.0	0.342	.....	.....	.....	.....
25.0	0.000	55.0	0.000	85.0	0.000	115.0	0.000	145.0	0.000	175.0	0.000

TABLE I—(*Continued*)

Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth	Height of growing zone	Rate of growth
Total height 35 cm.		Total height 65 cm.		Total height 95 cm.		Total height 125 cm.		Total height 155 cm.		Total height 195 cm.	
cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
8.3	0.000	23.8	0.000	56.4	0.000	91.3	0.000	128.0	0.000	176.8	0.000
12.0	0.130	30.0	0.097	64.0	0.109	95.0	0.045	132.0	0.060	180.0	0.068
16.0	0.324	35.0	0.204	69.0	0.227	100.0	0.128	136.0	0.154	183.0	0.118
20.0	0.523	42.0	0.382	74.0	0.375	105.0	0.267	140.0	0.305	185.0	0.260
22.0	0.610	47.0	0.523	78.0	0.530	108.0	0.379	143.0	0.432	187.5	0.460
24.0	0.666	51.0	0.620	82.0	0.630	113.0	0.602	145.0	0.543	.....	.....
26.0	0.690	54.6	0.670	85.2	0.652	115.9	0.634	147.3	0.615	189.2	0.590
28.0	0.638	57.7	0.616	87.0	0.616	117.0	0.616	149.0	0.600	191.0	0.533
30.0	0.544	49.0	0.554	89.0	0.560	119.0	0.575	151.0	0.523	193.0	0.430
32.0	0.410	61.0	0.470	91.0	0.470	121.0	0.500	153.0	0.400	.....	.....
33.0	0.331	63.0	0.325	93.0	0.330	123.0	0.340	.....	.....	.....	.....
35.0	0.000	65.0	0.000	95.0	0.000	125.0	0.000	155.0	0.000	195.0	0.000

stalks as closely comparable in size and behavior as it was possible to select from among those upon which measurements of growth rates were determined. These were collected between 9 and 10 A.M. and were cut about 2.5 cm. below the surface of the soil, taken to the laboratory, and cut transversely into segments of appropriate length. The length and position of the segments for stalks of different heights are given in table II. Branching usually began by the time stalks had reached a height of 100 cm. Where branches were present they were removed so that the samples consisted only of central axes.

In preparing the samples for analysis the segments were finely ground, duplicate 100-gm. samples weighed out, and 95 per cent. alcohol added in sufficient quantity to give a final concentration of 75 to 80 per cent. The material was then heated to boiling and the containers sealed and stored until the analyses were begun.

The nitrogen determinations were made upon separate samples which were dried rapidly in a current of warm air at 40° C. Total nitrogen was determined by the Gunning-Arnold method as modified to include nitrates.

The alcohol was decanted from the preserved material and passed through an extraction thimble. After adding alcohol twice and decanting each time, the residue was transferred to the extraction thimble which was then placed in a Soxhlet apparatus, and extracted about 8 hours with 95 per cent. alcohol to remove the last trace of alcohol-soluble materials. The extracts were combined, made up to volume, and aliquot portions taken for the determination of sugars, acids, and tannins. Sugars were determined by the volumetric permanganate modification of the Munson-Walker method (**1**).

The alcohol insoluble residue was dried, weighed, and duplicate portions taken for the estimation of the acid-hydrolyzable polysaccharides. These were determined by hydrolysis with hydrochloric acid as prescribed in the Official Methods (**1**).

Total astringency was determined by titration with N/20 potassium permanganate with indigocarmine as an indicator.

Total titratable acidity was determined by titration with N/10 sodium hydroxide with phenolphthalein as an indicator.

Experimental results

Rate of elongation along the stalk

Several observers have noted that the rate of growth along the stalk varies in different regions, and Working (**14**) has given a good account of this behavior. The data here presented are the averages of 116 stalks, and may be considered as representing the behavior of the field as a whole. Of course, the absolute rates will vary with the vigor of the plant, fertility of the soil,

the temperature and many other conditions. For a stalk of any given height the rate of growth at the apex is slow, but increases for a short distance downward from the apex to a maximum and then decreases again further toward the base to a point where it ceases. Table I gives the approximate height of the stalk at which it ceases to elongate, the height at which the rate is maximum, and the value of the maximum rate as well as a number of values at intermediate heights in the growing region, for plants 15 to 195 cm. in height. The progressive change in the rate of growth along the stalk is evident from the table but a clearer understanding of the manner of this change may be gained from figure 1. The values of the rates of growth

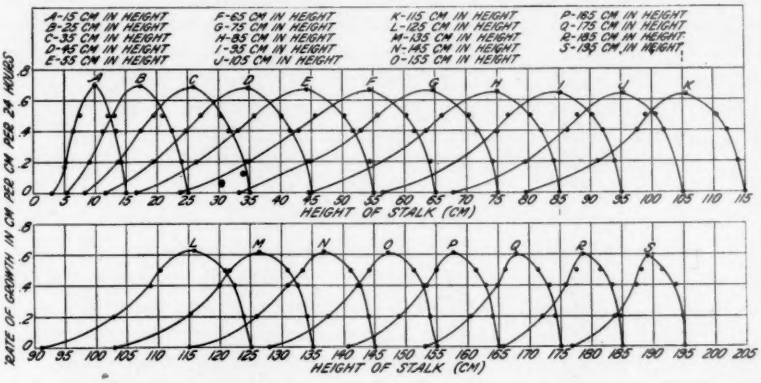
A-15 CM IN HEIGHT
B-25 CM IN HEIGHT
C-35 CM IN HEIGHT
D-45 CM IN HEIGHT
E-55 CM IN HEIGHT
F-65 CM IN HEIGHT
G-75 CM IN HEIGHT
H-85 CM IN HEIGHT
I-95 CM IN HEIGHT
J-105 CM IN HEIGHT
K-115 CM IN HEIGHT
L-125 CM IN HEIGHT
M-135 CM IN HEIGHT
N-145 CM IN HEIGHT
O-155 CM IN HEIGHT
P-165 CM IN HEIGHT
Q-175 CM IN HEIGHT
R-185 CM IN HEIGHT
S-195 CM IN HEIGHT
RATE OF GROWTH IN CM PER CM PER 24 HOURS
HEIGHT OF STALK (CM)
HEIGHT OF STALK (CM)

FIG. 1. The rate of growth of the stalks of asparagus at successive intervals from base to tip for stalks of various total heights.

represented by the ordinates are plotted against the corresponding heights of the growing segments of the stalk as abscissae for plants of many different total heights. A system of curves is thus formed, each curve of which represents the growth of a stalk of a particular height. Thus A represents the growth of a stalk 15 cm. high; B, one 25 cm. high; and so on at 10 cm. intervals until the stalk is 195 cm. in height. Each of the curves begins at a certain point on the abscissa at which the value of the ordinate is zero and ends at a point corresponding to the total height of the plant. Between the point on the abscissa at which the curve begins and the origin of the abscissa the rate of growth is obviously zero.

The curves show at once that the rate of growth in different zones along the stalk varies continuously from the lower point, where the growth ceases, to the tip. The form of the curve, however, is different for the plants of each particular height. For plants 15 cm. in height the curve rises rapidly to a maximum and then declines just as rapidly to the tip of the stalk. For those 65 to 75 cm. tall the curve rises gradually to a peak, then declines rapidly,

the decline being only a little less rapid than in the young stalks. For plants 195 cm. tall the curves rise gradually at first, then more sharply until a maximum is reached after which there is a rapid decline.

It is noted that the height of the point at which the stalk ceases to elongate is constantly being shifted upward as the plant becomes taller, but the rate does not parallel the rate of growth of the plant in height, being slower in the short, or young stalks, than in the high ones. The point of cessation of growth moves up the stalk slowly in the early stages of growth and then more rapidly as a height of 75 cm. or more is reached.

The point on the curve representing the zone of maximum rate of elongation shows that the rate of growth is maximum in a zone a short distance below the tip but this distance from the tip is not constant; it varies somewhat with the height of the stalk, being shortest in the youngest and the oldest stalks. The maximum rate of growth per unit length appears to be nearly constant for plants of all heights within the range studied. There is a tendency for the maximum rate to decline as the stalks become taller. The maximum is 0.7 cm. per cm. per 24 hours in stalks 15 cm. tall and 0.59 cm. per cm. for those 195 cm. tall.

It was pointed out in an earlier report (6) that the total amount of elongation occurring in 24 hours varies with the height of the stalk. Since it was found that the maximum rate of growth per unit length was nearly the same for stalks of all heights, it follows that differences in amount of elongation among stalks of different heights must be attributed for the most part to differences in the length of the portion that is elongating. The length of the elongating region varies with increasing height of the stalk, being about 11.7 cm. in stalks 15 cm. high; about 41.5 cm. in those 75 cm. high (in which it was at a maximum); and 18.3 cm. in those 195 cm. high. The height of the stalk in which the elongating region is of maximum length corresponds closely to that at which the total elongation is at a maximum, the most rapid rate of total elongation being in stalks 65 cm. high and the length of the growing region being maximum in those 75 cm. high.

Composition of the stalk at different heights

Variation in the growth rate along the stalks of asparagus raises the question as to what chemical changes are occurring in the different regions and how these are related to the growth rates. It was not feasible to make a sufficient number of replications of the analyses to procure enough data to permit curve smoothing as was done with the growth data, hence no attempt has been made to average the results.

The material used for analyses was necessarily taken from different stalks than those used in the growth measurements, but was as nearly identical as the writers could select. The possibility of a certain amount of sampling error, however, must be kept in mind.

Analyses were confined to the following constituents: total solids, sugars, total nitrogen, acids, tannins, and acid-hydrolyzable polysaccharides.

The results of the analyses are given in table II. The method of analyzing the growth data was not anticipated when the chemical samples were taken, and as a result the data for the composition and the data for the growth rates are not always recorded for stalks of identical heights or for identical positions on the stalk. Therefore, the relationship between the composition and the rate of growth is more easily comprehended if the curves obtained by plotting the data against the height of the segments are compared.

MOISTURE CONTENT AND GROWTH RATE

In figure 2 the percentage of total solids in successive segments along the

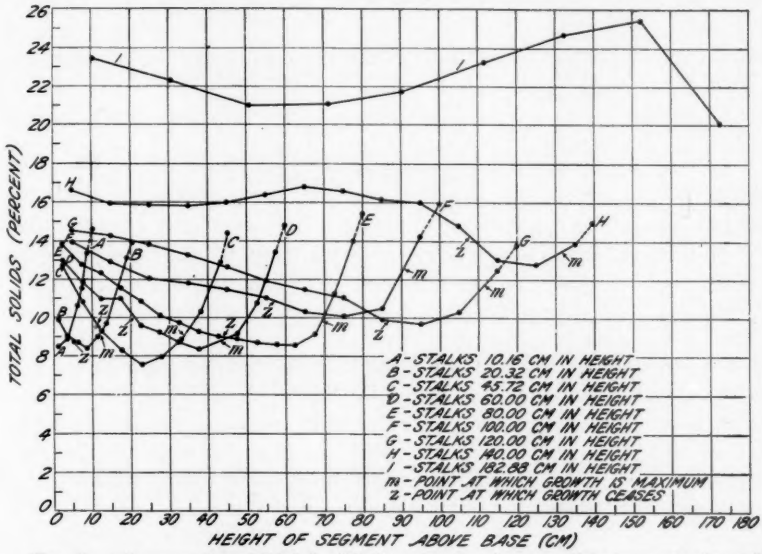
TOTAL SOLIDS (PERCENT)
HEIGHT OF SEGMENT ABOVE BASE (CM)
A - STALKS 10.16 CM IN HEIGHT
B - STALKS 20.32 CM IN HEIGHT
C - STALKS 45.72 CM IN HEIGHT
D - STALKS 60.00 CM IN HEIGHT
E - STALKS 80.00 CM IN HEIGHT
F - STALKS 100.00 CM IN HEIGHT
G - STALKS 120.00 CM IN HEIGHT
H - STALKS 140.00 CM IN HEIGHT
I - STALKS 182.88 CM IN HEIGHT
m - POINT AT WHICH GROWTH IS MAXIMUM
z - POINT AT WHICH GROWTH CEASES

FIG. 2. The percentage of total solids in asparagus in successive segments along the stalk for stalks of various heights.

stalk is plotted against the height of the segment above the base for stalks of various heights. It is evident that there is a great variation in the solids content of stalks of different height as well as in different regions along the stalk. Also, increase in height of the stalk is accompanied by a progressive increase in total solids. In stalks 10.1 cm. high the solids are lowest at the base and increase rapidly toward the tip. In stalks 20.3 cm. high the solids in the basal segments are somewhat higher than in the base of the 10.1 cm. stalks but they decrease along the stalk to a point about 9 cm. above

TABLE II

COMPOSITIONS OF THE ASPARAGUS STALK AT DIFFERENT POINTS ALONG THE STALK FROM BASE TO TIP FOR STALKS OF VARIOUS HEIGHTS. EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT

DATE OF SAMPLING	TOTAL HEIGHT OF STALK	LENGTH OF SEGMENT ANALYZED	MEAN HEIGHT OF SEGMENT FROM BASE	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	TOTAL SOLIDS	REDUCING SUGARS	NON REDUCING SUGARS	TOTAL SUGARS	ACID HYDROLYZABLE POLYSACCHARIDES	TOTAL ASTRINGENCY	TITRATABLE ACIDITY CALCULATED AS CITRIC
	cm.	*cm.*	*cm.*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*
May 4	10.16	2.4	1.27	5.72	2.77	8.49	3.05	1.05	4.10	0.69	0.076	0.227
" "	"	"	3.81	5.46	3.38	8.84	2.81	1.17	3.98	0.76	0.072	0.243
" "	"	"	6.35	5.36	5.23	10.59	2.16	1.14	3.30	0.98	0.100	0.255
" "	"	"	8.89	5.06	8.15	13.21	1.07	1.67	1.74	1.23	0.161	0.290
May 4	20.32	2.5	1.27	5.74	4.15	9.89	3.08	1.08	4.16	1.25	0.070	0.163
" "	"	"	3.81	5.40	3.65	9.05	2.98	0.24	3.92	0.95	0.074	.....
" "	"	"	6.35	5.16	3.57	8.73	2.94	0.86	3.80	0.91	0.084	0.185
" "	"	"	8.89	4.92	3.45	8.38	2.60	0.92	3.52	0.80	0.084	0.185
" "	"	"	11.43	4.94	4.05	8.99	2.48	0.64	3.12	0.86	0.104	0.227
" "	"	"	13.97	4.94	4.69	9.63	2.10	0.80	2.90	0.93	0.127	0.234
" "	"	"	16.51	4.92	6.27	11.19	1.57	0.85	2.42	1.00	0.151	0.286
" "	"	"	19.05	4.58	8.54	13.12	0.05	0.24	0.29	0.97	0.239	0.306
May 12	45.72	5	2.54	6.56	6.20	12.76	3.63	1.31	4.94	1.75	0.081	0.202
" "	"	"	7.62	5.98	4.82	10.80	3.39	1.28	4.68	1.30	0.082	0.189
" "	"	"	12.70	5.40	3.84	9.24	3.06	1.04	4.10	1.03	0.085	0.189
" "	"	"	17.78	4.94	3.32	8.26	2.87	0.69	3.56	0.85	0.081	0.174
" "	"	"	22.86	4.62	2.94	7.56	2.68	0.66	3.34	0.66	0.098	0.189
" "	"	"	27.94	4.56	3.39	7.95	2.43	0.73	3.16	0.75	0.096	0.228
" "	"	"	33.02	4.54	4.30	8.84	2.11	0.75	2.86	0.82	0.100	0.270
" "	"	"	38.10	4.60	5.68	10.28	1.58	0.66	2.24	0.86	0.144	0.274
" "	"	"	43.18	4.48	8.42	12.90	0.10	0.20	0.30	0.80	0.217	0.300

TABLE II—(*Continued*)

Date of sampling	Total height of stalk	Length of segment analyzed	Mean height of segment from base	Soluble solids	Insoluble solids	Total solids	Reducing sugars	Non reducing sugars	Total sugars	Acid hydrolyzable polysaccharides	Total astringency	Titratable acidity calculated as citric
	cm.	*cm.*	*cm.*	%	%	%	%	%	%	%	%	%
May 16	60	5	2.5	6.05	6.96	13.01	2.31	0.87	3.18	2.00	0.050	0.282
" "	"	"	7.5	5.43	6.38	11.81	2.46	0.63	3.09	1.81	0.046	0.282
" "	"	"	12.5	5.13	5.85	10.98	2.50	0.43	2.93	1.49	0.056	0.229
" "	"	"	17.5	5.11	5.88	10.99	2.16	0.73	2.89	1.35	0.064	0.261
" "	"	"	22.5	5.08	4.55	9.63	1.88	0.82	2.70	1.08	0.066	0.249
" "	"	"	27.5	5.28	3.96	9.24	2.21	0.41	2.62	0.98	0.076	0.260
" "	"	"	32.5	5.20	3.56	8.76	2.30	0.33	2.63	0.94	0.073	0.315
" "	"	"	37.5	4.93	3.39	8.32	2.11	0.33	2.44	0.87	0.080	0.315
" "	"	"	42.5	4.80	3.82	8.62	2.04	0.19	2.23	0.95	0.097	0.327
" "	"	"	47.5	4.80	4.37	9.17	1.73	0.44	2.17	0.84	0.104	0.352
" "	"	"	52.5	4.88	5.86	10.74	1.30	0.43	1.73	0.78	0.132	0.302
" "	"	"	57.5	4.82	8.61	13.43	0.20	0.29	0.49	0.74	0.184	0.324
May 17	80	5	2.5	5.80	8.03	13.83	2.87	0.60	3.47	2.85	0.063	0.261
" "	"	"	7.5	5.63	7.11	12.74	2.54	0.82	3.36	2.79	0.056	0.235
" "	"	"	12.5	5.68	6.62	12.30	2.64	0.70	3.34	2.51	0.059	0.236
" "	"	"	17.5	5.34	6.18	11.52	2.46	0.83	3.29	2.64	0.041	0.215
" "	"	"	22.5	5.19	5.63	10.82	2.04	0.86	2.90	2.48	0.061	0.253
" "	"	"	27.5	5.01	5.07	10.08	2.39	0.53	2.92	2.05	0.056	0.236
" "	"	"	32.5	4.95	4.79	9.74	2.37	0.41	2.78	1.97	0.054	0.245
" "	"	"	37.5	5.03	4.23	9.26	2.39	0.28	2.67	1.70	0.060	0.276
" "	"	"	42.5	4.94	4.09	9.03	2.21	0.54	2.75	1.35	0.054	0.251
" "	"	"	47.5	5.13	3.79	8.92	2.35	0.32	2.67	1.01	0.060	0.270
" "	"	"	52.5	4.94	3.80	8.74	2.30	0.28	2.58	0.98	0.062	0.256
" "	"	"	57.5	4.90	3.74	8.64	2.24	0.09	2.33	0.90	0.056	0.281
" "	"	"	62.5	4.58	4.02	8.60	2.13	0.20	2.33	0.94	0.072	0.296
" "	"	"	67.5	4.90	4.23	9.13	1.72	0.35	2.07	0.96	0.076	0.313
" "	"	"	72.5	4.87	6.40	11.27	1.34	0.33	1.67	1.02	0.115	0.285
" "	"	"	77.5	4.75	9.25	14.00	0.16	0.03	0.19	0.98	0.185	0.300

TABLE II—(*Continued*)

Date of sampling	Total height of stalk	Length of segment analyzed	Mean height of segment from base	Soluble solids	Insoluble solids	Total solids	Reducing sugars	Non reducing sugars	Total sugars	Acid hydrolyzable polysaccharides	Total astringency	Titratable acidity calculated as citric
	cm.	*cm.*	*cm.*	%	%	%	%	%	%	%	%	%
May 18	100	10	5	6.04	7.84	13.88	3.16	0.72	3.88	2.68	0.046	0.201
" "	"	"	15	5.66	7.21	12.87	2.95	0.61	3.56	2.89	0.056	0.218
" "	"	"	25	5.45	6.57	12.01	2.70	0.70	3.40	2.90	0.060	0.215
" "	"	"	35	5.26	6.54	11.80	2.74	0.45	3.19	2.00	0.072	0.242
" "	"	"	45	5.42	6.14	11.56	2.44	0.60	3.04	2.31	0.080	0.261
" "	"	"	55	5.48	5.61	11.09	2.56	0.53	3.09	1.82	0.088	0.305
" "	"	"	65	5.12	5.17	10.29	2.72	0.28	3.00	2.00	0.076	0.236
" "	"	"	75	5.71	4.41	10.12	2.59	0.25	2.84	1.56	0.074	0.260
" "	"	"	85	5.88	4.62	10.50	2.33	0.19	2.52	0.95	0.100	0.327
" "	"	"	95	5.96	8.28	14.24	1.27	0.38	1.64	0.86	0.283	0.330
May 23	120	10	5	5.96	8.57	14.53	3.26	0.48	3.74	3.11	0.046	0.196
" "	"	"	15	5.84	8.45	14.29	3.16	0.44	3.60	3.00	0.052	0.242
" "	"	"	25	6.40	7.43	13.83	2.77	0.74	3.51	3.21	0.052	0.249
" "	"	"	35	6.32	7.03	13.35	2.83	0.44	3.27	2.96	0.056	0.201
" "	"	"	45	6.16	6.49	12.65	2.68	0.42	3.10	2.74	0.062	0.268
" "	"	"	55	5.09	6.90	11.99	2.60	0.28	2.88	2.27	0.058	0.222
" "	"	"	65	5.25	6.25	11.50	2.50	0.33	2.83	1.95	0.062	0.322
" "	"	"	75	5.09	5.98	11.07	2.42	0.38	2.70	1.92	0.064	0.235
" "	"	"	85	5.64	4.34	9.98	2.18	0.47	2.65	1.99	0.060	0.252
" "	"	"	95	4.70	5.00	9.70	2.23	0.37	2.60	0.94	0.088	0.402
" "	"	"	105	5.42	4.85	10.27	2.19	0.26	2.45	0.92	0.100	0.392
" "	"	"	115	5.53	6.93	12.46	1.44	0.11	1.55	0.96	0.220	0.320

TABLE II—(*Concluded*)

Date of sampling	Total height of stalk	Length of segment analyzed	Mean height of segment from base	Soluble solids	Insoluble solids	Total solids	Reducing sugars	Non reducing sugars	Total sugars	Acid hydrolyzable polysaccharides	Total astringency	Titratable acidity calculated as citric
	cm.	*cm.*	*cm.*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*
June 8	140	10	5	5.50	11.10	16.60	3.07	0.75	3.82	2.31	0.040	0.252
" "	"	"	15	5.30	10.61	15.91	3.20	0.54	3.74	2.96	0.064	0.332
" "	"	"	25	5.76	10.06	15.82	2.18	0.52	3.70	3.00	0.076	0.356
" "	"	"	35	5.89	10.91	15.80	1.60	0.92	3.52	2.96	0.076	0.384
" "	"	"	45	5.60	10.41	16.01	1.78	0.72	3.50	2.85	0.091	0.376
" "	"	"	55	5.53	10.89	16.42	1.90	0.52	3.42	2.85	0.096	0.412
" "	"	"	65	5.51	10.30	16.81	1.57	0.73	3.30	2.92	0.104	0.404
" "	"	"	75	5.86	10.74	16.60	1.75	0.25	3.00	2.50	0.096	0.444
" "	"	"	85	5.68	10.50	16.18	1.56	0.26	2.82	2.71	0.120	0.450
" "	"	"	95	5.78	10.24	16.02	1.42	0.98	2.40	2.68	0.148	0.458
" "	"	"	105	5.72	10.10	14.82	1.36	0.65	2.01	2.47	0.144	0.444
" "	"	"	115	6.04	7.02	13.06	1.06	0.81	1.87	1.89	0.208	0.484
" "	"	"	125	6.44	6.36	12.80	1.00	0.48	1.48	1.78	0.280	0.430
" "	"	"	135	6.96	6.86	13.82	0.94	0.42	1.36	0.89	0.324	0.458
June 1	182.88	20.32	10.16	6.10	17.35	23.45	2.93	1.35	4.28	4.65	0.104	0.338
" "	"	"	30.48	5.80	16.54	22.34	2.68	1.20	3.88	4.17	0.141	0.342
" "	"	"	50.80	5.64	15.35	21.00	2.67	0.95	3.62	3.52	0.116	0.377
" "	"	"	71.12	5.64	15.44	21.08	2.34	1.02	3.36	3.54	0.128	0.393
" "	"	"	91.44	5.38	16.99	21.77	2.35	0.73	3.08	3.65	0.134	0.404
" "	"	"	111.76	5.12	18.12	23.24	2.34	0.72	3.06	3.99	0.128	0.443
" "	"	"	132.08	5.20	19.49	24.69	1.95	0.77	2.72	4.25	0.145	0.455
" "	"	"	152.40	5.04	20.39	25.43	1.64	0.80	2.44	4.61	0.172	0.500
" "	"	"	172.72	5.64	15.08	20.72	1.05	0.29	1.34	2.64	0.486	0.491

the base, then increase rapidly to the tip. In stalks 45.7 cm. tall the curve is distinctly U-shaped, the lowest solids content being about 23 cm. from the base, or about 20 cm. below the tip. In stalks of all heights up to 150 cm. the solids are lowest in some portion intermediate between the base and the tip. The zone of low solids is constantly being shifted upward as the stalks become taller. The solids content increases rapidly in the basal portion of the stalks as they increase in height; likewise segments immediately above the base continue to increase in solids content so that the length of the basal portion with a high solids content is constantly increasing. This results in a high solids content all along the stalks as they approach maturity. The curves are thus constantly changing form as the height of the stalk increases, being U-shaped in the young, then dipper-shaped, and finally more or less horizontal in the mature stalks.

It may be estimated from figure 2 that in stalks 60 cm. tall, the zone of elongation begins at about 20.5 cm. and reaches a maximum at about 49.5 cm. from the base, and as indicated in figure 3, curve d, the lowest solids content is found approximately 37 cm. from the base. Growth ceases at Z (fig. 2), and is at a maximum at M. The highest moisture content is found just above the point at which growth ceases and just below the point at which growth rate is at a maximum. In stalks 80 cm. tall the lower limit of growth is 38.5 cm. above the base and growth is maximum at about 69.5 cm. The highest moisture content is about 56 cm. above the base. The moisture content, then, is highest neither in the region in which growth is at its maximum nor at the lower limit of the growing region. If the two sets of stalks used for measurements of growth rate and chemical analysis are comparable, it must be concluded that the cell walls begin to thicken sufficiently to give a higher solids content in the stalk before the cells wholly cease to elongate.

It is evident that hydration is an important factor in the elongation of the stalk. At the tip of the stalk, cell division is very rapid but the cells enlarge slowly at first probably because the mechanism of water transport is as yet imperfectly formed. As soon as water is brought to the cells in quantity they enlarge very rapidly but soon after the transporting system is sufficiently formed to function adequately for the enlarging cells, its structural elements gradually become rigid and elongation ceases. These processes are constantly going on so that the region of activity is constantly being shifted upward.

Soluble and insoluble solids

The proportion of soluble to insoluble solids differs greatly in different zones along the stalk and varies somewhat in stalks of different heights. It may be noted from table II that the soluble solids are less variable than the insoluble. There is a tendency for the soluble solids to be highest at the base and lowest at the tip or in a zone somewhat below the tip. The difference in

the percentage of soluble solids between the old and young stalks is not very great and appears unimportant. The insoluble solids content changes very greatly from base to tip, being lowest in a zone between the region of maximum rate of growth and the lower limit at which growth occurs. At the base the insoluble solids are evidently composed very largely of structural materials and vary greatly in amount with the height of the stalk or the age of the tissues. The insoluble solids at the tip are obviously high in protein and their amount does not vary as widely as the insoluble content at the base.

Total sugars and growth rate

The results of the sugar analyses show (fig. 3) that the sugar content is, in general, fairly low, and varied considerably in the different series of

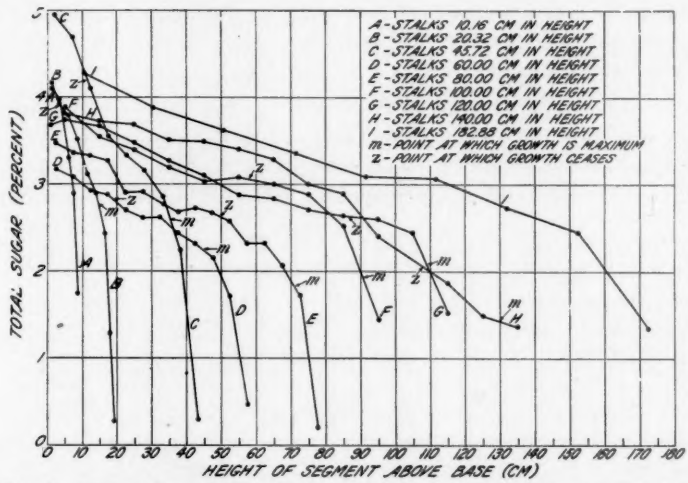
A - STALKS 10.16 CM IN HEIGHT
B - STALKS 20.32 CM IN HEIGHT
C - STALKS 45.72 CM IN HEIGHT
D - STALKS 60.00 CM IN HEIGHT
E - STALKS 80.00 CM IN HEIGHT
F - STALKS 100.00 CM IN HEIGHT
G - STALKS 120.00 CM IN HEIGHT
H - STALKS 140.00 CM IN HEIGHT
I - STALKS 182.88 CM IN HEIGHT
m - POINT AT WHICH GROWTH IS MAXIMUM
z - POINT AT WHICH GROWTH CEASES
TOTAL SUGAR (PERCENT)
HEIGHT OF SEGMENT ABOVE BASE (CM.)
0
1
2
3
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180

Fig. 3. Percentage of total sugar in asparagus in successive segments along the stalks of various heights.

stalks. This variation is probably due to sampling error, to differences in crown reserves, or to some variation in weather conditions at the time the samples were taken. The sugar content varied tremendously in different regions along the stalk, always being highest at the base and lowest at the tip. Sugar is apparently almost absent at the extreme tip of the growing stalk. The sugar content in the terminal segment of the stalks above 80 cm. in height is somewhat higher than in the terminal segments of the short stalks, probably because the segment analyzed was much longer and consequently included tissues far enough down the stalk to be somewhat higher in sugar than at the tip. The concentration gradient is that to be expected

where sugar is being transported from the root to the growing tip if diffusion plays a part in the process. As the stalks become taller the basal portion that is high in sugar increases in length so that the content at any specific height is constantly changing in an elongating stalk.

The letter M (fig. 3) indicates the region at which the growth is at a maximum and the letter Z the lower limit at which elongation occurs. It may be noted that there is a very sharp decrease in the sugar at about the region where growth is at a maximum. The very rapid use of sugar for the formation of cellulose and other structural substances and the diluting effect of the absorbed water probably account for its very low concentration in this region. FERNALD (7) has found that the freezing point depression is maximum at the tip, decreases for a distance below the tip, and then becomes somewhat irregular; it generally increases again toward the basal end of the stalk. This does not seem to be related to the sugar content, but to a certain extent parallels the total solids content. Several factors are evidently concerned.

TOTAL NITROGEN

The percentage of total nitrogen in successive segments along the stalk plotted against the height of the segment above the base is shown in figure 4. It is apparent that the percentage of nitrogen is high at the tip and low

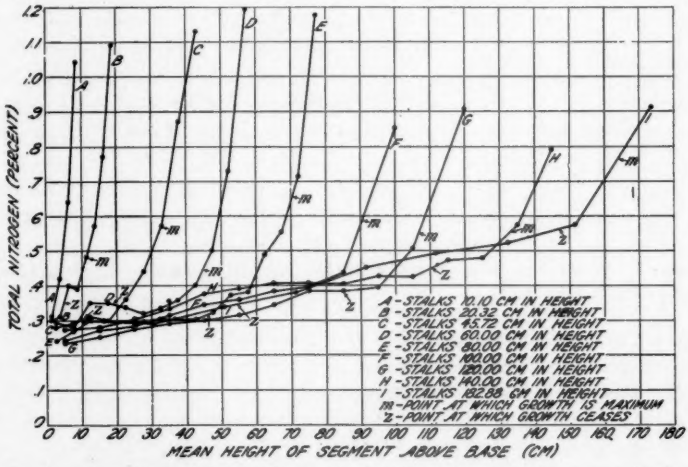
TOTAL NITROGEN (PERCENT)
MEAN HEIGHT OF SEGMENT ABOVE BASE (CM)
A - STALKS 10.10 CM IN HEIGHT
B - STALKS 20.32 CM IN HEIGHT
C - STALKS 45.72 CM IN HEIGHT
D - STALKS 60.00 CM IN HEIGHT
E - STALKS 80.00 CM IN HEIGHT
F - STALKS 100.00 CM IN HEIGHT
G - STALKS 120.00 CM IN HEIGHT
H - STALKS 140.00 CM IN HEIGHT
I - STALKS 182.88 CM IN HEIGHT
M - POINT AT WHICH GROWTH IS MAXIMUM
Z - POINT AT WHICH GROWTH CEASES

FIG. 4. Percentage of total nitrogen in asparagus in successive segments along the stalks of varfious heights.

in the basal portion of stalks of all heights. In young stalks 10.1 cm. tall the percentage of nitrogen increases rapidly from base to tip; the same is true for those 20.3 cm. tall to a somewhat smaller degree. In stalks 60 cm.

tall the nitrogen is fairly low for about 20 cm. above the base, then rises rapidly to a maximum at the tip. In those 100 cm. tall, it rises slowly to about 80 cm. in height and then increases rapidly to the tip. The region of maximum growth corresponds approximately to that at which the percentage of nitrogen begins its rapid rise. In other words, the points of inflection on the curves correspond very roughly to zones in which growth is at a maximum. Below these zones the curves are markedly flat. It is also evident that in the zone where growth ceases the percentage of total nitrogen has fallen to a low level. Also from the very high moisture content at this point it may be inferred that the protoplasm has become greatly vacuolated or has taken into its confines large amounts of water which lowers the total nitrogen content on the fresh weight basis.

The percentage of total nitrogen in the segments at the tip remains surprisingly near the same value in the tall as in the short stalks. The results are somewhat irregular and the values tend to decrease slightly in the taller stalks. The irregularity is probably due in part to sampling error while the tendency to decrease is probably the result of analyzing a longer segment, and of a relative decrease in nitrogen supply.

The question arises as to how the stalk maintains a high percentage of nitrogen at the tip in spite of its rapid elongation. This may be explained by the transportation of soluble nitrogen from the roots to the apical portion of the stalk before and shortly after it emerges from the soil. Up to this time increase in length has been relatively slow but increase in diameter has been comparatively rapid. By the time the stalk is 10 to 20 cm. tall, it has almost reached its full diameter at the surface of the soil and large amounts of nitrogenous substances have accumulated in the region from the apex downward for some distance.

In table III is given the fresh weight, the percentage of total nitrogen on the fresh weight basis, and the total weight of the nitrogen in 1-cm. sections of the fresh stalk at different heights. The fresh weight and chemical data were secured from different sets of stalks. For this reason the total weight of nitrogen per cm. of stalk may be subject to considerable sampling error but every effort was made to select comparable material.

It is noted in table III that the weight of the apical section decreases as the stalks become taller. In stalks 10.16, 20.32, 60, and 80 cm. in height, the weights of the tip segments were 1.21, 1.01, 0.706, and 0.563 gm. respectively. In the same way the segment immediately below the tip decreased in weight with an increase in height of the stalks and so on with the third and fourth segment below the tip.

The absolute weight of nitrogen also decreases in the tip segment as the stalk increases in height. In stalks 10.16, 60, and 80 cm. high, the tip centimeter has 0.0126, 0.011, and 0.0066 gm. of nitrogen respectively. In the

same way the segment immediately below the tip decreases in its absolute amount of nitrogen as the stalk becomes higher. In general the same ten-

TABLE III

FRESH WEIGHT AND TOTAL NITROGEN CONTENT OF 1-CM. SEGMENTS OF ASPARAGUS STALKS AT DIFFERENT HEIGHTS FROM THE BASE

TOTAL HEIGHT OF STALK	HEIGHT OF SEGMENT ABOVE BASE	FRESH WEIGHT OF 1-CM. SEGMENT OF STALK	TOTAL NITROGEN FRESH WEIGHT BASIS	WEIGHT OF NITROGEN IN 1-CM. SEGMENT OF STALK
cm.	*cm.*	*gm.*	%	*gm.*
10.16	1.27	2.40	0.31	0.00744
"	3.81	2.33	0.42	0.00978
"	6.35	2.12	0.64	0.01356
"	8.89	1.21	1.04	0.01260
20.32	1.27	2.78	0.30	0.00735
"	3.81	2.52	0.31	0.00781
"	6.35	2.19	0.40	0.00876
"	8.89	2.18	0.42	0.00916
"	11.43	2.00	0.48	0.00955
"	13.97	1.75	0.57	0.00995
"	16.51	1.48	0.77	0.01140
"	19.05	1.01	1.09	0.01100
45.72	2.54	2.71	0.28	0.00760
"	7.62	2.67	0.29	0.00776
"	12.70	2.42	0.31	0.00749
"	17.78	2.53	0.30	0.00760
"	22.86	2.15	0.36	0.00775
"	27.94	1.81	0.44	0.00795
"	33.02	1.51	0.57	0.00862
"	38.10	1.16	0.87	0.01010
"	43.18	0.88	1.13	0.00991
60	2.5	2.49	0.294	0.00732
"	7.5	2.65	0.282	0.00749
"	12.5	2.53	0.301	0.00758
"	17.5	2.21	0.350	0.00773
"	22.5	2.18	0.338	0.00735
"	27.5	2.23	0.321	0.00715
"	32.5	2.03	0.335	0.00682
"	37.5	1.93	0.359	0.00692
"	42.5	1.76	0.405	0.00715
"	47.5	1.55	0.500	0.00778
"	52.5	1.19	0.739	0.00882
"	57.5	0.706	1.195	0.00840
80	2.5	2.96	0.241	0.00714
"	7.5	2.70	0.267	0.00720
"	12.5	2.33	0.310	0.00730
"	17.5	2.36	0.306	0.00722
"	22.5	2.51	0.300	0.00754
"	27.5	2.03	0.296	0.00700
"	32.5	2.17	0.304	0.00662
"	37.5	1.96	0.302	0.00593
"	42.5	1.92	0.304	0.00584

TABLE III—(*Continued*)

FRESH WEIGHT AND TOTAL NITROGEN CONTENT OF 1-CM. SEGMENTS OF ASPARAGUS STALKS AT DIFFERENT HEIGHTS FROM THE BASE

TOTAL HEIGHT OF STALK	HEIGHT OF SEGMENT ABOVE BASE	FRESH WEIGHT OF 1-CM. SEGMENT OF STALK	TOTAL NITROGEN FRESH WEIGHT BASIS	WEIGHT OF NITROGEN IN 1-CM. SEGMENT OF STALK
cm.	*cm.*	*gm.*	%	*gm.*
"	47.5	1.77	0.324	0.00573
"	52.5	1.57	0.371	0.00585
"	57.5	1.48	0.380	0.00562
"	62.5	1.17	0.487	0.00570
"	67.5	1.19	0.553	0.00638
"	72.5	1.15	0.713	0.00695
"	77.5	0.563	1.177	0.00663
100	5	2.66	0.240	0.00638
"	15	2.62	0.270	0.00710
"	25	2.41	0.295	0.00710
"	35	2.13	0.317	0.00675
"	45	1.95	0.349	0.00667
"	55	1.66	0.360	0.00600
"	65	1.34	0.385	0.00518
"	75	1.08	0.397	0.00425
"	85	0.82	0.435	0.00357
"	95	0.50	0.853	0.00426
120	5	2.71	0.244	0.00661
"	15	2.68	0.251	0.00672
"	25	2.52	0.277	0.00694
"	35	2.23	0.295	0.00658
"	45	1.92	0.304	0.00584
"	55	1.75	0.316	0.00553
"	65	1.40	0.347	0.00486
"	75	1.16	0.387	0.00449
"	85	0.94	0.385	0.00375
"	95	0.69	0.396	0.00273
"	105	0.40	0.506	0.00202
"	115	0.30	0.905	0.00271
140	5	2.62	0.274	0.00710
"	15	2.60	0.278	0.00723
"	25	2.45	0.300	0.00735
"	35	2.20	0.337	0.00748
"	45	1.92	0.376	0.00723
"	55	1.68	0.392	0.00658
"	65	1.40	0.407	0.00570
"	75	1.19	0.406	0.00483
"	85	1.02	0.401	0.00409
"	95	0.80	0.426	0.00341
"	105	0.61	0.421	0.00257
"	115	0.44	0.470	0.00207
"	125	0.33	0.573	0.00189
"	135	0.26	0.759	0.00197

dency is evident in the third and fourth segment below the tip. A more complete picture of the nitrogen trends is shown in figure 5. The absolute

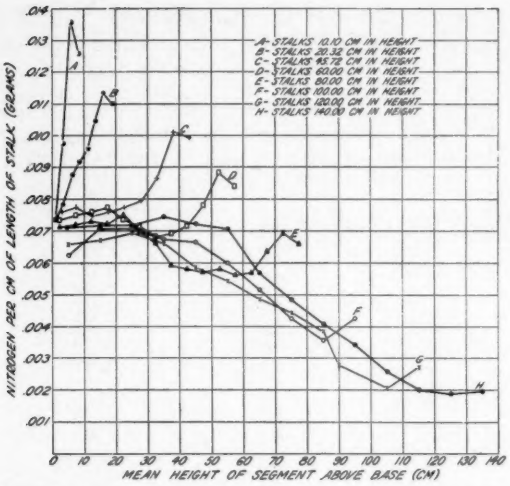

FIG. 5. The absolute amount of nitrogen per cm. length of the stalk of asparagus in successive segments from base to tip.

amount along the stalk also varies from base to tip and is greatest near the tip, particularly in stalks up to 60 cm. in height. In stalks above 80 cm. in height there is a tendency for the absolute amount to decrease along the stalk from the base to the region of the growing zone when it again increases to the tip. This is owing to the difference in the total weight of the segments of different heights and to the percentage of nitrogen present. Being a monocotyledonous plant, the diameter of the stem becomes fixed by morphological limitations a few centimeters below the tip.

It is well known that through the activity of the protoplasm or the nitrogenous substances the formation of the structural materials is effected. The cells when first formed at the apex are densely filled with protoplasm and a short distance below the tip they begin a rapid extension in size by the absorption of water which is then followed by a thickening and further differentiation of the structural elements that continues until the stem is fully mature. Thus a small amount of nitrogenous substances in the apical portion of the stalk may suffice to form a large amount of structural tissues and the reserve supply in the tip appears to be used in this way. With these facts in mind it is not surprising that the plant is able to maintain the percentage of nitrogen in the tip at a rather high value during the period of most rapid elongation.

TITRATABLE ACIDITY

The titratable acidity is lowest in the basal segments and highest near the tip (table II). The higher acidity near the tip may be caused by a greater

amino acid content in this region. No tests were made to determine the active acidity, or hydrion concentration, but it closely parallels the titratable acidity in many plant materials. CALDWELL (**4**) has shown that in the growth of many young fruits the rapid increase in size and hydration of the tissues of the fruit is accompanied by an increased active acidity. The acidity was generally highest in the tip segment; but in stalks 60 and 80 cm. tall it was highest in approximately the region of maximum growth rate. More work is necessary to establish the character of the acidity gradient in the growing stalks.

TANNIN CONTENT

The substances which account for the values here obtained are known only in part for there is no doubt that substances other than true tannins enter into the reactions. These substances vary considerably in amount in the tissues of the stalks at different heights. They are always lowest toward the base and highest near the tip. Their distribution along the stalk is shown in figure 6. The percentage present in any region appears to be closely

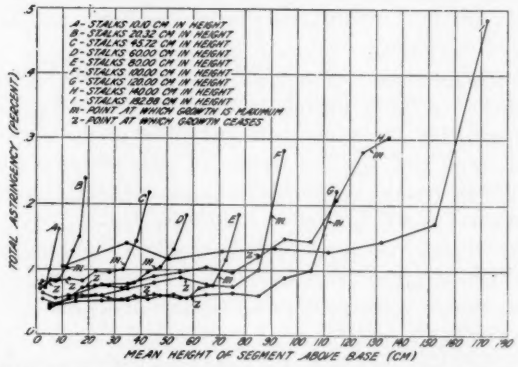
A - STALKS 10.10 CM IN HEIGHT
B - STALKS 20.32 CM IN HEIGHT
C - STALKS 45.72 CM IN HEIGHT
D - STALKS 60.00 CM IN HEIGHT
E - STALKS 80.00 CM IN HEIGHT
F - STALKS 100.00 CM IN HEIGHT
G - STALKS 120.00 CM IN HEIGHT
H - STALKS 140.00 CM IN HEIGHT
I - STALKS 182.88 CM IN HEIGHT
III - POINT AT WHICH GROWTH IS MAXIMUM
Z - POINT AT WHICH GROWTH CEASES
TOTAL ASTRINGENCY (PERCENT)
MEAN HEIGHT OF SEGMENT ABOVE BASE (CM)

FIG. 6. The percentage of total astringency in asparagus in successive segments along the stalks of various heights.

correlated with the total nitrogen. They are high in amount during the early growth processes but decrease to a low level below the point on the stalk where growth ceases. They also appear to be higher in the green portion of the stalk than in the portion with little green color present.

ACID-HYDROLYZABLE POLYSACCHARIDES

According to TANRET (**11**) starch does not occur in the asparagus plant but inulin-like substances are present instead. It is apparent from table II that the acid-hydrolyzable polysaccharides are generally very low. Although the values appear somewhat erratic there is a tendency for these

substances to be lowest in the region between the points of maximum growth and cessation of growth. Hydrolysis with hydrochloric acid probably destroys some of the levulose which makes the results somewhat irregular.

Summary

1. Using field grown material, the rate of elongation of the stem of asparagus in different zones along the stem was determined for plants of widely different heights at temperatures between 65 and 70° F. and the results plotted. The rates of elongation vary in a characteristic manner with each height of stalk. The zone of maximum growth changes its position relative to the tip with each height of stalk; the length of the growing region and the rate of growth in this region varies with each height of the plant. The rates of elongation in the zones of maximum growth are nearly constant over a rather wide range of heights. There is a tendency for this maximum value to decrease somewhat with increase in height for stalks from 15 to 195 cm. high. The difference in the total elongation per 24 hours among stalks of different heights is caused principally by the difference in the length of the growing region and not by differences in the maximum rate of growth per unit length of growing zone.

2. Analyses made of segments of the stalk at different heights show that the total solids vary greatly at different points along the stalk. The total solids were lowest in a zone several centimeters below the tip, varying somewhat with the total height of the stalk. In stalks of intermediate height the curves representing the relationship are distinctly U-shaped, the lowest total solids content being found in the middle portion.

It appears that the lowest solids or the highest moisture is in a region between that at which growth is at a maximum and the point where it ceases. It is concluded that a certain amount of thickening of the cell walls occurs before the growth finally ceases.

3. Total sugars are highest at the base and decrease rapidly to a low value near the tip. They decrease very rapidly in the growth zone and above the point of maximum rate of elongation they decrease to a very low value. This may be due to the rapid use of the sugars in cellulose formation. The amount at any point is determined partly by the rate at which they are used, and partly by the rapidity with which they may be transported from the source of supply to the region where they are being used.

4. Total nitrogen is lowest in the basal portion of the stalk but increases rapidly in amount in regions near the tip. Total nitrogen or the density of the protoplasm constantly changes as growth development occurs in the growing region. The elongation of the stalk is accompanied by hydration of the cells which apparently continues until the cell walls become sufficiently

rigid to check the process. At the point where growth ceases the total nitrogen has decreased to a low value. In the region immediately above and below the point where growth ceases there is a rapid addition to the stalk of cellulose and other structural materials. In spite of these processes total nitrogen remains high at the extreme tip of the stalk. The total amount per unit length of the stalk, however, in the region near the tip does decrease as growth proceeds.

5. Changes in total astringency are very closely correlated with changes in the total nitrogen. The total astringency is lowest towards the base and highest at the tip.

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FOLIAR DIAGNOSIS: PHYSIOLOGICAL BALANCE BETWEEN THE BASES LIME, MAGNESIA, AND POTASH[1]

WALTER THOMAS AND WARREN B. MACK

(WITH FOUR FIGURES)

Introduction

One of the functions of calcium is to act as a base in neutralizing the acids of the tissues (**5**). Although other bases take part in the structure and functioning of plants, in terrestrial plants two bases, magnesia and potash, are of such importance as always to be employed in studies of plant nutrition. In certain respects each of these base-forming elements appears to fill a specific rôle; but considered as bases, functioning solely in their rôle of neutralizing the acids of the tissues, CaO, MgO, and K_2O are able chemically to replace one another equivalent for equivalent. If the plant selects one rather than another, it is the result of its normal or natural power to regulate the absorption of solutes. The object of this investigation is to examine the nature of this regulation.

In a cosmos regulated by immutable laws it would be logical to expect that the absorption of mineral elements would conform to a definite system, as evidence of which may be cited the fact that when growth is optimum the absorption of nitrogen and phosphoric acid is a linear function with time (**2, 6**).

In the studies on the method of leaf diagnosis thus far carried out (**7, 8, 9, 10**), calcium and magnesium were not determined because of the use (on the potato) of calcium-containing sprays. The fact was emphasized, however, that the basic assumption in fertilizer field plot experiments is that the factors other than the ones being investigated are nearly equal for the plots to be compared (**7**). A rigid examination of the effect of lime should accordingly comprise plots with and without lime for all treatments investigated.

In our investigations of the leaf, the determination of calcium and magnesium was first made on *Zea mays* growing on tier 1 of the Jordan fertility plots. In the following year the inclusion of Ca and Mg in the foliar diagnosis of the potato was made possible by omitting calcium-containing sprays.

Foliar diagnosis of maize

METHODS

PLOTS EXAMINED WITH THEIR TREATMENT.—The general plan of the Jordan Fertility experiments has been published by NOLL (**2**). The soil

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is formed by the disintegration of limestone rocks. In some of the plots, notably nos. 1–5, the parent rock is near the surface. Only two of the plots on tier 1 on which the present experiments were conducted had received lime; the other plots had not received any lime since 1881 (**11, 12**).

The results reported in this paper are those from plants growing on plots receiving single element treatments, combination of two elements, and also of all three elements. In addition a plot receiving manure and lime, and another lime alone and also an unfertilized (nothing) plot were included. The plots with treatments and yields are listed in table I.

TABLE I

PLOT TREATMENTS WITH YIELDS OF MAIZE

PLOT	SYMBOL	TREATMENT	YIELDS OF GRAIN PER PLOT
			lb.
1	Nothing	No lime	165.4
2	N	No lime, N as dried blood	265.6
3	P	No lime, P as superphosphate	371.2
4	K	No lime, K as muriate	298.3
5	NP	No lime, N as dried blood P as superphosphate	376.7
6	NK	No lime, N as dried blood K as muriate	263.5
7	PK	No lime, P as superphosphate K as muriate	497.4
9	NPK	No lime, N as dried blood P as superphosphate K as muriate	520.2
26	NPK	No lime, N as sodium nitrate P as superphosphate K as muriate	705.5
22	Manure + lime	6 tons of manure 2 tons of lime every 4 years	770.8
23	Lime	2 tons of lime every 4 years	452.2

LEAF SAMPLING.—The third leaf from the base was sampled in the manner previously described (**7, 9**) at four periods—*viz.*, July 6, July 21, August 8, and August 25. On two plots, check and manure (the lowest and highest yielding plots on this tier) samples were taken also on June 16.

PRESENTATION OF RESULTS

DERIVATION OF THE Ca–Mg–K–UNIT.—The results of the analysis for CaO, MgO, and K_2O in the third leaf are shown in table II (columns 4, 5, and 6). Inasmuch as the chemical reactions within the leaf are under consideration, these percentages of CaO, MgO, and K_2O in the dried foliage are converted into milligram equivalents (E_x, E_y, E_z, columns 7, 8, and 9). The last three columns show the values of the composite *Ca–Mg–K–unit*, which

TABLE II

PERCENTAGES OF CaO, MgO AND K_2O IN THE DRIED FOLIAGE OF MAIZE, THEIR MILLIGRAM EQUIVALENT VALUES, AND THE COMPOSITION OF THE *Ca-Mg-K-unit*

DATE OF SAMPLING	PLOT	TREATMENT	PERCENTAGE IN DRIED FOLIAGE			MILLIGRAM EQUIVALENT			COMPOSITION OF THE Ca–Mg–K–UNIT		
			CaO (M_x)	MgO (M_y)	K_2O (M_z)	CaO (E_x)	MgO (E_y)	K_2O (E_z)	X $\left(100 \cdot \frac{E_x}{S}\right)$	Y $\left(100 \cdot \frac{E_y}{S}\right)$	Z $\left(100 \cdot \frac{E_z}{S}\right)$
			%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*			
June 16			2.510	0.910	2.500	89.607	45.318	53.250	47.300	23.090	29.610
July 6			2.308	0.934	2.387	82.396	45.513	50.843	46.095	25.461	28.443
July 21	1	Check	2.087	0.966	2.515	74.506	48.107	53.570	42.289	27.305	30.406
Aug. 8			1.605	1.039	1.841	57.299	51.742	39.213	38.649	34.901	26.450
Aug. 25			1.999	1.216	1.608	71.364	60.557	34.250	52.946	36.443	20.611
July 6			2.058	1.177	2.248	73.471	58.615	47.882	40.824	32.570	26.606
July 21	2	N	2.435	1.448	2.104	86.929	72.110	44.815	42.643	35.373	21.984
Aug. 8			2.328	1.376	1.453	83.110	68.525	30.949	45.519	37.531	16.950
Aug. 25			2.222	1.159	1.481	79.325	57.718	31.545	47.053	34.236	18.711
July 6			3.131	1.901	1.019	111.777	94.670	21.705	48.992	41.494	9.513
July 21	3	P	3.744	2.462	0.756	133.611	122.608	16.103	49.073	45.015	5.912
Aug. 8			3.348	1.847	0.794	119.524	91.981	16.912	52.327	40.269	7.404
Aug. 25			2.937	1.792	0.911	104.851	89.242	19.404	49.111	41.800	9.089
July 6			1.690	0.815	3.984	60.333	40.587	84.859	32.476	21.847	45.677
July 21	4	K	1.887	1.112	4.000	67.366	55.378	85.200	32.396	26.631	40.973
Aug. 8			1.689	0.905	3.901	60.297	45.069	83.091	31.995	23.915	44.090
Aug. 25			1.491	0.742	2.480	53.229	36.952	52.824	37.222	25.840	36.938
July 6			3.410	2.114	0.724	121.737	105.277	15.421	50.214	43.424	6.362
July 21	5	NP	3.322	2.516	0.813	118.595	125.296	17.317	45.403	47.967	6.630
Aug. 8			3.322	2.077	0.813	118.595	100.944	17.317	50.072	42.619	7.309
Aug. 25			2.984	2.281	0.775	106.528	113.593	16.507	45.019	48.004	6.976
July 6			1.543	0.634	4.031	55.085	31.573	85.860	31.930	18.301	49.769
July 21	6	NK	1.510	0.833	4.209	53.907	41.483	89.652	29.132	22.418	48.450
Aug. 8			1.628	0.905	3.081	58.120	45.069	65.625	34.428	26.697	38.874
Aug. 25			1.309	0.525	2.763	46.731	26.145	58.852	35.475	19.848	44.677

TABLE II—(*Continued*)

Date of sampling	Plot	Treatment	Percentage in dried foliage			Milligram equivalent			Composition of the Ca–Mg–K–unit		
			CaO (M_x)	MgO (M_y)	K_2O (M_z)	CaO (E_x)	MgO (E_y)	K_2O (E_z)	X $\left(100 \cdot \frac{E_x}{S}\right)$	Y $\left(100 \cdot \frac{E_y}{S}\right)$	Z $\left(100 \cdot \frac{E_z}{S}\right)$
			%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*			
July 6	7	PK	1.902	0.605	3.565	67.901	30.129	75.936	39.032	17.319	43.649
July 21			2.221	0.829	3.508	79.290	41.284	74.720	40.600	21.139	38.260
Aug. 8			2.283	0.688	2.887	81.503	34.262	61.493	45.980	19.329	34.691
Aug. 25			1.917	0.507	2.655	68.437	25.249	56.551	45.553	16.806	37.641
July 6	9	NPK (N as dried blood)	1.720	0.606	3.674	61.404	30.179	78.256	36.154	17.769	46.077
July 21			1.887	0.707	3.766	67.366	35.209	80.216	36.854	19.262	43.884
Aug. 8			1.842	0.362	2.992	65.759	18.028	63.730	44.577	12.221	43.202
Aug. 25			1.705	0.271	2.879	60.869	13.496	61.323	44.860	9.946	45.194
July 6	26	NPK (N as sodium nitrate)	1.933	0.471	3.837	69.008	23.456	81.728	39.616	13.466	46.918
July 21			2.313	0.670	3.643	82.574	33.366	77.596	42.666	17.240	40.090
Aug. 8			2.237	0.453	2.946	79.861	22.559	62.750	48.351	13.658	37.991
Aug. 25			2.207	0.525	2.267	78.790	26.145	48.287	51.422	17.063	31.514
June 16	22	manure + lime	2.410	0.540	2.710	86.037	26.892	57.723	50.245	15.600	34.155
July 6			2.793	0.630	2.740	99.710	31.374	58.362	52.632	16.561	30.807
July 21			3.160	0.717	2.752	112.812	35.707	58.618	54.463	17.238	28.299
Aug. 8			3.381	0.760	2.092	120.702	37.848	44.560	59.427	18.634	21.939
Aug. 25			3.514	0.851	1.890	125.449	42.600	40.400	60.395	20.105	19.500
July 6	23	lime	4.144	1.376	0.872	147.941	68.525	18.574	62.943	29.155	7.902
July 21			4.233	1.231	0.910	151.118	61.304	19.383	65.192	26.446	8.362
Aug. 8			3.851	1.122	0.910	137.481	55.876	19.383	64.624	26.265	9.111
Aug. 25			3.983	1.014	0.891	142.193	50.497	18.978	67.177	23.857	8.966

represents the equilibrium between CaO–MgO–K_2O at the moment of sampling, and is derived by determining the proportion each of the milligram equivalent values for CaO, MgO, and K_2O bears to the total. Hence $\frac{E_x}{S}+\frac{E_y}{S}+\frac{E_z}{S}=1$. To avoid fractions, the magnitudes so obtained are multiplied by 100; therefore $\left(100\,\frac{E_x}{S}\right)+\left(100\,\frac{E_y}{S}\right)+\left(100\,\frac{E_z}{S}\right)=100$, or $X+Y+Z=100$. The values of $X+Y+Z$ for any given sample are the composite *Ca–Mg–K–unit* as defined above.

Discussion and interpretation of results

Relation between CaO–MgO–K_2O under optimum growth conditions is a linear function.—The *Ca–Mg–K–units* given in the last three columns of table II are plotted in trilinear coordinates in figure 1. The numerals 0, 1, 2, 3, 4 indicate the dates of sampling, June 16 (t = 0), July 6, July 21, August 8, August 25, respectively; on graphs without these numerals, the arrow with the treatment symbol indicates the first date (July 6), and others follow in order. As already stated, samples on June 16 (t = 0) were taken only from plants growing on the check and manure plots.

The broken line ee′ joins the coordinate points for samples taken July 6

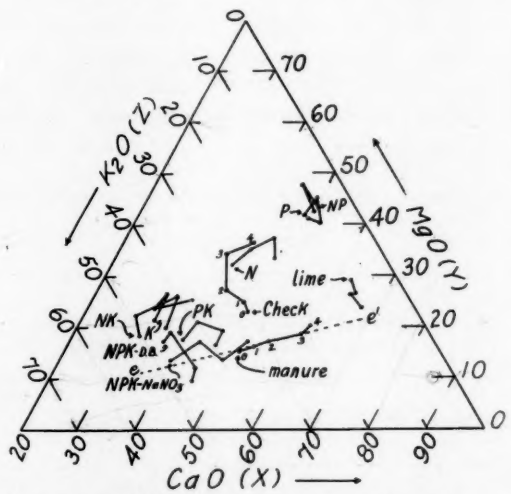

Fig. 1. Deviations from the optimum physiological balance (the line ee′) between CaO, MgO, and K_2O resulting from single "element," combination of two "elements," and a complete fertilizer carrying nitrogen in the two forms dried blood (D.B.) and sodium nitrate ($NaNO_3$) (maize experiments).

and August 8 from the maximum yielding plot (manure + lime). Equations of this line may be expressed in two ways, *viz.*, $ax + by + cz = 0$, where x, y, and z are the perpendicular distances drawn from the coordinate points to the sides of the triangle, and a, b, and c are constants; or in the standard form of the equation of a straight line $y = mx + b$.

Because of the fact that a line drawn from any point within an equilateral triangle perpendicular to one of the sides bears a constant relation to a line drawn from the same point to the same side, but parallel to one of the other two sides, it may be shown that the ordinates X, Y, and Z representing the *Ca–Mg–K–unit* may be substituted for x, y, and z of the first equation shown above. The equation of the line ee′ in trilinear coordinates, therefore, may be written in the form $aX + bY + cZ = 0$, or $X + \left(\frac{b}{a}\right)Y + \left(\frac{c}{a}\right)Z = 0$, and with the equation in this form it is possible to determine the values of $\left(\frac{b}{a}\right)$ and $\left(\frac{c}{a}\right)$ from the coordinates of any two points on the straight line. If the *Ca–Mg–K–units* for manure on July 6 (52.632: 16.561: 30.807) and on August 9 (59.427: 18.634: 21.939) are taken as the coordinates of these two points, the values of $\left(\frac{b}{a}\right)$ and $\left(\frac{c}{a}\right)$ are found to be -3.2079 and 0.0161, respectively. The equation of the line ee′ in trilinear coordinates, therefore, is $X - 3.2079Y + 0.161Z = 0$.

With observed values of MgO and K_2O at the different dates of sampling as Y and Z, the values of CaO at the respective dates may be calculated from the foregoing equation. The observed (X) and calculated (X_1) values thus computed and the ratio of the difference between them to the observed values $\left(\frac{X - X_1}{X}\right)$ are given in table III.

The simplest form of equation, and the one easiest to interpret physiolog-

TABLE III

EXPERIMENTAL VALUES AND VALUES CALCULATED FROM THE EQUATION IN TRILINEAR COORDINATES REPRESENTING THE EQUILIBRIUM AMONG CaO, MgO, AND K_2O AT FIVE SUCCESSIVE DATES OF SAMPLING FOR PLANTS OF *Zea mays* GROWING ON THE MANURE PLOT

DATES OF SAMPLING	OBSERVED VALUES OF CaO (X)	CALCULATED VALUES OF CaO (X_1)	DIFFERENCE, $\frac{X - X_1}{X}$
June 16	50.245	49.493	0.015
July 6	52.632	52.632	0.0
July 21	54.463	54.842	−0.007
Aug. 8	59.427	59.427	0.0
Aug. 25	60.395	64.182	−0.062

ically, is the linear one $Y = mX + b$, where X is the abscissa (CaO), and Y the ordinate (MgO). In this form the equation of the line ee′ is $Y = 0.305\,X + 0.050$. It is seen that with the exception of the values for the last sampling date, when chlorophyll degeneration had already begun, all the other values for this manure + lime plot (no. 22) lie sensibly on this line ee′. Since $X + Y + Z = 100$, and since $Y = mX + b$, it follows also that any two of these variables X, Y, Z are related by a linear equation.

The equation connecting the values for CaO (X) and K_2O (Z) is $Z = -1.305X + 99.490$ and that relating the values for MgO (Y) and K_2O (Z) is $Z = -0.233\,Y + 23.760$.

DEVIATION OF EXPERIMENTAL FROM CALCULATED VALUES.—The deviation of the experimental values from the values calculated from these equations is given in table IV. The values given in the last column represent the relative deviation of the milligram equivalent values, which are in the same proportion to each other as the values X, Y, and Z of the *Ca–Mg–K–units*.

It is seen that with the exception of the last sampling date the experimental values deviate very little from the calculated values.

TABLE IV

EXPERIMENTAL VALUES AND VALUES CALCULATED FROM THE THREE EQUATIONS REPRESENTING THE EQUILIBRIUM BETWEEN CaO–MgO, CaO–K_2O, AND K_2O–MgO AT FIVE SUCCESSIVE DATES OF SAMPLING FOR PLANTS OF *Zea mays* GROWING ON THE MANURE PLOTS

DATES OF SAMPLING	EXPERIMENTAL VALUES		CALCULATED VALUES	RELATIVE DEVIATION
	MgO (Y)	CaO (X)	CaO (X)	$\frac{X - X_1}{X}$
June 16	15.600	50.245	49.493	+0.014
July 6	16.561	52.632	52.632	0.0
July 21	17.238	54.463	54.865	−0.008
Aug. 8	18.634	59.427	59.427	0.0
Aug. 25	20.105	60.395	64.262	−0.063
	K_2O (Z)	CaO (X_2)	CaO (X_1)	$\frac{X - X_2}{X}$
June 16	34.155	50.245	50.065	+0.003
July 6	30.807	52.632	52.632	0.0
July 21	28.299	54.463	54.865	−0.004
Aug. 8	21.939	59.427	59.427	0.0
Aug. 25	19.500	60.395	61.226	−0.013
	MgO (Y)	K_2O (Z)	K_2O (Z_1)	$\frac{Z - Z_1}{Z}$
June 16	15.600	34.155	35.021	−0.025
July 6	16.561	30.807	30.807	0.0
July 21	17.238	28.299	28.000	+0.010
Aug. 8	18.634	21.939	21.939	0.0
Aug. 25	20.105	19.500	15.691	+0.190

SIGNIFICANCE OF THE EQUATIONS.—These equations show that as the values for CaO increase with maturity the values for MgO also become larger and by regular increments. On the other hand as the values for CaO increase, those for K_2O *decrease* regularly. Similarly, as the values for K_2O decrease those for MgO *increase* regularly also.

These relationships are of interest in relation to their bearing on observations by earlier workers which hitherto were incompletely understood. LOEW (**4**) concluded that plants require a definite CaO/MgO ratio in their medium, and that this requirement varied with the plant species. This hypothesis was succeeded by EHRENBERG's so-called "lime-potash law" (**1**) according to which lime reduces the amount of potassium taken up by plants. Later WIEGNER and MÜLLER (**13**) were able to show that the concentration of potassium in the aqueous phase of the soil is proportional to the sum of the potassium and calcium dissolved from the absorbing soil complex.

Although the amounts of nutrient and other materials in the plant are determined by the quantities available, the absorption of elements is not in the proportion in which they exist in the soil, but in proportions modified by certain characteristics of the plant (**5**). The equilibrium between CaO–MgO, between CaO–K_2O, and between MgO–K_2O indicated by the above equations shows the relative proportion in which they are present in the leaf during the growth cycle of *Zea mays*. When one or another of the elements is not present in sufficient amounts in the soil, the plant responds by a relatively excess absorption of one of the others, resulting in a disequilibrium which is reflected in reduced yields.

The seat of the inhibition of the absorption of potassium by calcium then lies in the soil and not within the plant.

DEVIATIONS FROM EQUILIBRIUM CONDITIONS WITH CHARACTERISTICS OF THE GRAPHS OF THE DIFFERENT TREATMENTS.—The deviations from equilibrium conditions resulting from the other treatments are shown in figure 1. The graphs are compared with that of the highest yielding treatment (manure + lime, plot 22), represented by the line ee′.

The characteristics of these graphs: *Check (no. 1)* [no fertilizer, no lime].—Relative to the treatment highest yielding, manure + lime (plot 22) the original unfertilized soil contains (with respect to *Zea mays*) great excess (relatively) of MgO, deficiency of CaO, and sufficient K_2O.

Nitrogen (no. 2) [no lime, no phosphoric acid, and no potash].—Deficiency (relative) of CaO, great excess of MgO, and slight deficiency of K_2O.

Phosphorus (no. 3) [no lime, no nitrogen, no potash].—The displacement towards the right side of the triangle and high up towards the summit indicates great excess (relative) of MgO, slight deficiency of CaO, and great deficiency of K_2O.

Potash (no. 4) [no lime, no nitrogen, no phosphorus].—The displace-

ment is well over to the left side of the triangle indicating great deficiency (relative) of CaO and excess of K_2O and also of MgO.

Nitrogen and phosphorus (no. 5) [no lime, no potash].—Slight deficiency (relative) of CaO becoming greater with maturity, great excess of MgO, and great deficiency of K_2O.

Nitrogen and potash (no. 6) [no lime, no phosphorus].—Great deficiency (relative) of CaO, and excess of K_2O and also of MgO.

Phosphorus + potash (no. 7) [no lime, no nitrogen].—Relative deficiency of CaO, and excess of K_2O and of MgO.

Nitrogen + phosphorus + potash (no. 9) [no lime, nitrogen as dried blood].—Deficiency of CaO, excess of K_2O, and variable MgO, which is too high at first and with advancing age of the leaf becomes too low.

Nitrogen + phosphorus + potash (no. 26) [no lime, nitrogen as sodium nitrate].—In comparison with no. 9 (N as dried blood), sodium nitrate has increased the CaO and reduced the K_2O, but has changed the MgO but little.

Lime (no. 23) [no nitrogen, no phosphoric acid, and no potash].—Great deficiency (relative) of potash, great excess of CaO, and slight excess of MgO.

In [P], [NP], and [lime] particularly, and to a lesser extent in [check] and [N], then, there is relative insufficiency of K_2O. In these cases the demand of meristematic tissues results in export of potassium, which is normally necessary, from the leaf and accumulation therein of an abnormal proportion of CaO and MgO in the place of the K_2O. The leaf then is exhausted cell by cell until finally it dies. This degeneracy of the leaf was evident towards the latter part of July in [P], [NP], [check], and [N].

Foliar diagnosis of the potato

Methods

The inclusion of calcium and magnesium in the investigations on the leaf diagnosis of the potato was first carried out in 1937, on section D of the vegetable fertility plots (**7, 9, 10**). These plots had been limed with approximately four-fifths of the quantity of ground high-calcium limestone calculated to meet the theoretical lime requirement as determined by a modified Veitch method. The plants were sprayed weekly with cuprous oxide suspended in water by means of a neutral wetting agent, at the rate of two pounds of cuprous oxide to 100 gallons of water. Considerable damage was done to the plants by a severe attack of green apple leafhoppers, which were not controlled by the addition of an aliphatic thiocyanate insecticide (Lethane 440) which was included regularly in the cuprous oxide spray according to manufacturers' directions after the leafhoppers appeared.

The attack was of such severity that by August 20th no living leaves older than the 8th from the bottom could be found on plants on the manure plots.

On other plots damage was evident, but the lower leaves had not fallen off to such an extent as they had on the manure plots.

THE PLOTS EXAMINED, WITH THEIR TREATMENTS.—The plots with treatment examined in this paper are given in table V.

TABLE V

PLOTS STUDIED, WITH TREATMENTS AND YIELDS OF TUBERS

TIER	PLOT	TREATMENT	SYMBOL	YIELDS PER PLOT
		lb.		*lb.*
1	2	3.75 lb. $NaNO_3$	N	36.0
1	3	6.25 superphosphate (16%)	P	43.6
1	4	1.60 muriate (50%)	K	49.7
1	6	3.75 $NaNO_3$ and 6.25 superphosphate	NP	50.3
1	7	3.75 $NaNO_3$ and 1.60 muriate	NK	48.1
1	8	6.25 superphosphate and 1.60 muriate	PK	76.6
1	10	3.75 $NaNO_3$ and 6.25 superphosphate and 1.60 muriate	NPK	105.0
2	4	5.63 $NaNO_3$ and 6.25 superphosphate and 1.60 muriate	(1.5N)PK	67.0
2	8	3.75 $NaNO_3$ and 9.38 superphosphate and 1.60 muriate	N(1.5P)K	84.5
2	12	3.75 $NaNO_3$ and 6.25 superphosphate and 2.40 muriate	NP(1.5K)	90.1
2	15	5.63 $NaNO_3$ and 9.38 superphosphate and 2.40 muriate	1.5(NPK)	74.8
1	15	600 Manure	Manure	124.0

LEAF SAMPLING.—The fourth leaf from the base was sampled on July 2, July 16, August 5, in the manner previously recorded (7). Samples were taken also on August 20, but at this date leaves varying from the 6th to the 8th were the oldest available.

PRESENTATION OF RESULTS

Table VI shows the quantities of CaO, MgO, and K_2O in the dried foliage expressed as (1) percentages, (2) milligram equivalents, and (3) the *Ca–Mg–K–unit*.

INTERPRETATION AND DISCUSSION OF RESULTS

CONSEQUENCES OF THE ATTACK BY LEAF HOPPERS.—In some plots (especially the manure plots) the first 8 leaves from the base were dead by August 20, and it was not possible to secure healthy leaves lower than the

TABLE VI

PERCENTAGES OF CaO, MgO, AND K_2O IN THE DRIED FOLIAGE FROM POTATO PLANTS TOGETHER WITH THEIR MILLIGRAM EQUIVALENT VALUES AND THE COMPOSITION OF THE *Ca-Mg-K-unit*

DATE OF SAMPLING	PLOT	TREATMENT	PERCENTAGE IN DRIED FOLIAGE			MILLIGRAM EQUIVALENT			COMPOSITION OF THE Ca–Mg–K–UNIT		
			CaO (M_x)	MgO (M_y)	K_2O (M_z)	CaO (E_x)	MgO (E_y)	K_2O (E_z)	X $\left(100\ \frac{E_x}{S}\right)$	Y $\left(100\ \frac{E_y}{S}\right)$	Z $\left(100\ \frac{E_z}{S}\right)$
			%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*			
July 2	1–2	N	2.960	1.249	3.353	105.672	62.150	71.419	44.17	25.97	29.85
July 16			3.626	1.521	3.364	129.448	75.746	71.653	46.75	27.36	25.88
Aug. 5			4.536	1.839	3.353	160.935	91.582	71.419	49.68	28.27	22.04
Aug. 20			4.032	1.810	2.209	143.942	90.138	47.052	51.20	32.06	16.73
July 2	1–3	P	4.225	1.014	3.023	150.832	50.497	64.390	56.76	19.00	24.23
July 16			5.320	1.112	2.240	189.924	55.377	47.712	64.81	18.89	16.29
Aug. 5			7.000	1.940	1.628	249.900	96.612	34.676	65.56	25.34	9.09
Aug. 20			5.570	1.860	1.465	198.849	91.827	31.204	61.77	28.22	10.00
July 2	1–4	K	2.360	0.652	5.562	84.252	32.469	118.470	35.82	13.80	50.37
July 16			2.772	0.669	5.833	98.960	33.316	124.244	38.57	12.98	48.44
Aug. 5			3.752	0.744	5.232	113.946	36.951	109.311	43.79	14.20	42.00
Aug. 20			3.710	0.706	4.624	132.447	35.158	98.491	49.77	13.21	37.01
July 2	1–6	NP	4.490	1.187	2.848	160.029	59.112	60.662	57.22	21.12	21.65
July 16			5.796	1.593	1.918	206.917	79.331	40.853	63.25	24.25	12.49
Aug. 5			5.726	1.738	1.508	204.418	86.552	32.120	63.27	26.78	9.94
Aug. 20			4.676	1.955	1.124	166.933	97.359	23.941	57.91	33.77	8.31
July 2	1–7	NK	2.630	0.985	6.345	93.891	49.053	118.875	35.86	18.73	45.40
July 16			2.940	1.086	5.891	104.958	54.082	125.073	36.96	19.03	44.02
Aug. 5			3.696	1.303	5.348	131.947	60.617	107.330	43.99	20.21	35.79
Aug. 20			3.892	1.285	4.922	138.944	63.993	95.764	46.51	21.42	32.06
July 2	1–8	PK	3.400	0.579	5.213	121.380	28.834	121.367	44.69	10.61	44.69
July 16			4.210	0.669	5.058	150.297	33.316	117.235	49.95	11.11	38.93
Aug. 5			5.016	0.687	4.554	179.071	34.212	107.330	55.85	10.67	33.47
Aug. 20			4.860	0.669	4.263	173.502	33.316	70.566	62.54	12.01	25.44

TABLE VI—(*Continued*)

Date of sampling	Plot	Treatment	Percentage in dried foliage			Milligram equivalent			Composition of the Ca–Mg–K–unit		
			CaO (M_x)	MgO (M_y)	K_2O (M_z)	CaO (E_x)	MgO (E_y)	K_2O (E_z)	X $\left(100\ \frac{E_x}{S}\right)$	Y $\left(100\ \frac{E_y}{S}\right)$	Z $\left(100\ \frac{E_z}{S}\right)$
			%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*			
July 2	1–10	NPK	4.032	0.677	6.589	143.942	33.714	127.139	47.22	11.06	41.71
July 16			5.586	0.869	5.794	199.420	43.276	123.412	54.47	11.87	33.65
Aug. 5			5.922	1.028	4.360	211.415	51.194	98.661	58.51	14.16	27.32
Aug. 20			5.530	1.129	3.871	197.421	56.224	82.963	58.65	16.70	24.64
July 2	2–4	(1.5N)PK	3.320	0.641	6.527	118.522	31.922	124.264	43.14	11.62	45.23
July 16			4.382	0.779	6.124	156.437	38.794	137.044	47.08	11.67	41.24
Aug. 5			4.858	0.807	5.620	173.430	40.188	114.743	52.81	12.23	34.96
Aug. 20			4.760	0.887	4.573	169.932	44.172	103.198	53.53	13.92	32.54
July 2	2–8	N(1.5P)K	3.724	0.753	5.457	132.946	37.499	137.385	43.18	12.18	44.63
July 16			4.956	0.916	4.767	176.693	45.616	128.375	50.38	13.00	36.61
Aug. 5			5.530	1.086	5.271	197.421	54.082	96.169	56.84	15.55	27.60
Aug. 20			5.640	1.122	4.205	201.348	55.875	73.463	60.88	16.89	22.22
July 2	2–12	NP(1.5K)	3.360	0.554	7.085	119.952	27.589	145.308	40.96	9.42	49.61
July 16			4.354	0.651	6.725	155.138	32.419	146.544	46.43	9.70	43.86
Aug. 5			5.152	0.851	4.418	183.926	42.379	127.949	51.93	11.97	36.09
Aug. 20			4.620	0.896	5.019	164.934	43.276	106.073	52.48	13.77	33.74
July 2	2–15	1.5(NPK)	3.556	0.641	6.201	126.949	31.922	146.373	41.58	10.46	47.95
July 16			4.396	0.709	5.930	156.937	35.308	140.345	40.71	10.61	48.67
Aug. 5			4.718	0.832	5.290	168.432	41.433	118.044	51.36	12.63	36.00
Aug. 20			4.900	0.807	4.225	174.930	40.188	103.177	54.96	12.62	32.41
July 2	1–15	Manure	3.360	1.133	6.066	119.952	56.423	134.552	38.58	18.14	43.27
July 16			4.396	1.303	5.839	156.937	64.889	119.706	45.95	18.99	35.05
Aug. 5			5.300	1.219	5.020	189.210	60.678	107.707	53.01	17.07	30.00
Aug. 20			3.976	1.104	5.194	141.943	54.979	110.732	46.13	17.87	35.99

sixth from the base on any of the plots at this date. Inasmuch as the basis of sampling for leaf diagnosis lies in the fact that leaves from morphologically homologous positions on the stalk are the seat of the same physiological process, leaves taken at the last sampling (August 20) are considered as being in the same physiological conditions as the fourth leaf at an earlier date. This assumption, however, will not prevent comparison being made with the maximum yielding plot (manure, no. 15).

EQUATION OF EQUILIBRIUM BETWEEN CaO–MgO–K_2O FOR THE MAXIMUM (BUT NOT OPTIMUM) TREATMENT.—In figures 2 to 4 are plotted in trilinear coordinates the *Ca–Mg–K–unit* previously defined for the fourth leaf of plants growing on the plots indicated.

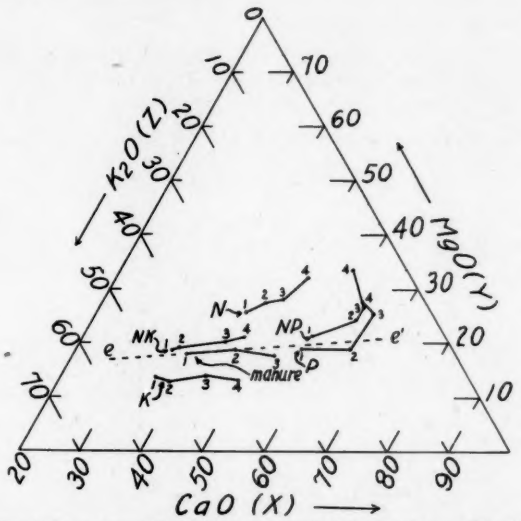

FIG. 2. Deviations from the optimum physiological balance (the line ee′) between CaO, MgO, K_2O resulting from the treatments indicated (potato experiments).

The broken line ee′ is drawn through the coordinate values for July 2 and July 16 of the highest yielding plot—*viz.*, manure (no. 1–15). The line joining the coordinate points for July 2 and July 16 has been extended and taken as the evolutionary course of the CaO–MgO–K_2O equilibrium of the fourth leaf during the growth cycle. The assumption is made that this would represent the course of nutrition as represented by the *Ca–Mg–K–units* if the plants had not been arrested in development at about August 20th as the result of unexpected contingencies, since we have shown that under optimum conditions, the relationship among CaO, MgO, and K_2O is definitely linear in the case of *Zea mays*. The general conclusions made below will not be affected by this assumption.

Using the same coordinate nomenclature as before—*viz.*, ***CaO = (X), MgO = (Y), and K_2O = (Z)***, the equation of this line for manure (no. 1–15) is $Y = 0.115X + 13.69$.

CHARACTERISTICS OF THE GRAPHS OF THE *Ca–Mg–K–units* FOR THE VARIOUS TREATMENTS.—The treatments may be grouped as follows according to their position with respect to the line ee′; that is, comparison is made with the graph of the maximum yielding plot (manure, plot no. 15).

Group 1.—NK (no. 1–7), K (no. 1–4) (figure 2): are distinguished from all other treatments in that these are the only two treatments that result in relative excess of K_2O in the *Ca–Mg–K–unit*. The displacement is slightly to the left (below ee′ in [K] and above in [NK], indicating a small deficiency (relative) of CaO and a slight excess of K_2O. A deficiency of MgO exists in [K] and an excess in [NK].

Group 2.—N (no. 1–2), P (no. 1–3), NP (no. 1–5) (figure 2): Displacement is to the right, indicating excess (relative) of CaO and deficiency of K_2O. In [P] and [NP], balance with respect to MgO nearly exists in the young leaf, but the disequilibrium becomes greater and greater with advancing age.

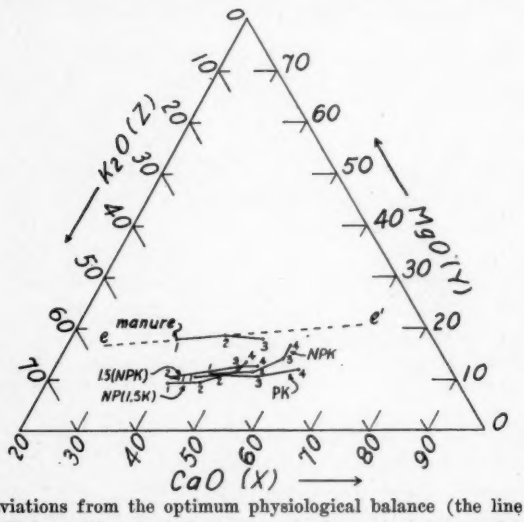

FIG. 3. Deviations from the optimum physiological balance (the line ee′) between CaO, MgO, and K_2O resulting from the treatments shown (potato experiments).

Group 3.—NPK (no. 1–10), PK (no. 1–7), (figure 3): Displacement is towards the right base apex and below the line ee′, indicating in the *Ca–Mg–K–unit* deficiency of MgO and slight excess of CaO.

Group 4.—NP (1.5K) (no. 2–12), 1.5 (NPK) (no. 2–15) (figure 3): Displacement is towards the right base apex and below the line ee′, indicating in

the *Ca-Mg-K-unit* deficiency of MgO and slight excess of CaO and slight deficiency of MgO and slight excess of CaO and slight deficiency of K_2O.

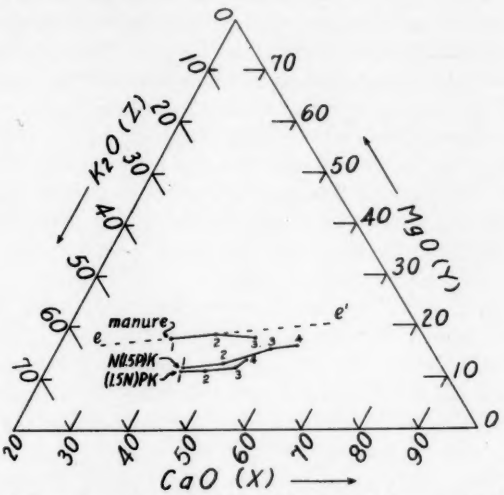

FIG. 4. Deviations from the optimum physiological balance (the line ee′) between CaO, MgO, K_2O resulting from the [N(1.5P)K] and [(1.5N)PK] treatments (potato experiments).

Group 5.—N(1.5P)K (no. 2–8); (1.5N)PK (no. 2–4) (figure 4). Displacement is to the right and below ee′, indicating in the *Ca-Mg-K-unit* deficiency of MgO, with slight excess of CaO.

DEPRESSING EFFECT OF CALCIUM ON THE ABSORPTION OF POTASH.—The positions of the graph of [P] and [NP] in figures 1 and 2, *i.e.*, of treatments containing superphosphate without potash indicates the poverty in K_2O of the *Ca-Mg-K-unit* of the chosen leaf resulting from these treatments, showing that the effect of calcium in superphosphate cannot be ignored in field studies (**12**).

BEARING OF THESE RESULTS ON STUDIES IN HUMAN AND ANIMAL NUTRITION.—The results of this study suggest also that attempts to classify foods into acid-forming and base-forming from the analysis of the ash requires a knowledge of the conditions under which the plants were grown before any generalization with respect to a particular plant is possible.

Summary

In this mysterious universe the planetary system of which is governed by laws that can be given mathematical expression, it is logical to hypothecate that the physiological processes of living organisms follow laws that

await discovery. In the normally functioning plant the relationship between nitrogen and phosphoric acid has been shown to be a linear function in time (**6**). The results of the present investigation indicate the law governing the absorption of the principal bases, lime, magnesia, and potash is, under optimum conditions, a linear function also. The experiments were at first conducted with maize growing on the Jordan fertility plots and subsequently with the potato growing on the vegetable fertility plots of the Pennsylvania Agricultural Experiment Station. In the former the third leaf and in the latter the fourth leaf from the base was sampled periodically during the growth cycle, following the method described earlier (**7**).

The equilibrium between CaO-MgO-K_2O on any sampling date is expressed as a composite *Ca-Mg-K unit* derived after the manner of the *NPK-unit* (**7**)–*viz.*, the percentage composition for CaO, MgO, and K_2O respectively at any given date is converted into milligram equivalent values and the proportion which each of these bears to the milligram equivalent total determined. The values so obtained are multiplied by 100 to avoid fractional values. The *Ca-Mg-K-units* so obtained are plotted in trilinear coordinates.

In the case of maize the line joining the coordinate points for July 6 to August 8 for the maximum yielding plot (manure + lime) is sensibly rectilinear in time. The deviations of the experimental values and the values calculated from the linear equation are given.

The significance of the equations relating the absorption of the three variables CaO, MgO, and K_2O is discussed and their bearing on the work of earlier investigators is described.

The characteristics of the graphs for other plot treatments unlimed since 1881, together with a plot receiving lime only are discussed in relation to their deviation from the optimum graph (manure + lime).

The plots of the vegetable fertility experiments, in contrast, had received applications of ground limestone in amounts calculated to bring all the plots to approximately the same pH values. As a result the deviation from a linear function between the three bases is less marked for most treatments in the potato experiments. In the former the following treatments did not cause great deviation from the linear relationship: [K], [NK], [PK], [1.5N(PK)], [N(1.5P)K], [NP(1.5K)], and [1.5(NPK)]; nevertheless their position (as distinguished from form) relative to the graph of the highest yielding plot indicates that disequilibrium exists between CaO-MgO-K_2O in the plots. The characteristics of the graphs in relation to the graph from the highest yielding treatment, manure, are described.

The evolutionary sequence of the composite *Ca-Mg-K-unit* constitutes a sensitive and faithful diagnosis of the mode of adaptation of the medium (soil) to the needs of the plant with respect to CaO, MgO, and K_2O, when the respective graphs are interpreted in relation to the optimum treatment.

The bearing of the results on studies in human and animal nutrition is indicated.

LABORATORY OF PLANT NUTRITION
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EFFECTS OF CERTAIN INSECTICIDES AND INERT MATERIALS UPON THE TRANSPIRATION RATE OF BEAN PLANTS[1]

E. C. WAGNER

(WITH SEVEN FIGURES)

Introduction

The changes in physiological processes of plants brought about by the application of spray materials are not well understood, nor have they been investigated in detail. That such changes do occur is evidenced by both the beneficial and the harmful effects of these compounds quite apart from their value as fungicides or insecticides. The increased growth and greater yield of potatoes sprayed with Bordeaux, the shot-hole effect of copper fungicides on peach leaves, the russeting of fruit and burning of foliage by arsenicals all point to profound changes in the physiology of the sprayed tissues.

In an attempt to determine the basis for the relative "safeness" of certain arsenates as compared with others, investigations into the effects of both kinds of sprays on the physiological processes in plants have been made. The process most widely studied in this connection has been transpiration. Some writers have suggested that the phytocidal action of certain sprays might be attributed, at least in part, to the sudden increase in the rate of water loss from the sprayed plants. Thus it was thought that perhaps a so-called "safe" compound which did not cause the typical burning of sprayed leaves, was innocuous because it did not bring about the increase in water loss which resulted when the injurious sprays were applied to the leaves.

Not all investigators are agreed as to the effect of sprays on transpiration. Many have recorded results in which there was no increase, but a decrease, or no change at all in the rate of water loss. In fact, it has been suggested that the stimulatory effect of Bordeaux on potatoes may be attributed to a conservation of the water supply.

The earlier workers investigated the effect of Bordeaux on crop plants. In general, their results showed a decrease in the transpiration rate of the sprayed plants as compared with that of the controls. Many of the data, however, were not very quantitative.

Some time later, DUGGAR and COOLEY (3), in studies of cut plants in potometers, found that Bordeaux increased the transpiration rate of castor bean, squash, Phytolacca, potato, and Iresine. They also studied the rates

[1] Papers from the Department of Botany no. 406, the Ohio State University. This work was done in cooperation with the Sherwin-Williams Co., and the Ohio State University Research Foundation.

of water loss from potted plants of potato and tomato with the same results. The tomato plants did not show an increase in transpiration resulting from the application of calcium hydroxide, clay, aluminum hydroxide, calcium carbonate, or lime-sulphur 1–25, but the potato plants lost more water when sprayed with lime water, Bordeaux and lampblack, lampblack alone, lime-sulphur, and lime wash, than did the control plants. Lime-sulphur did not cause an increase in water loss.

MARTIN (**8**) reported that data from potometer experiments with leaves of radish, bean, swiss chard, Hibiscus, Clerodendron, Caladium, Datura, and castor bean, showed that spraying with Bordeaux brought about a rapid increase in the transpiration rate, which later tended to return to the original rate. In some experiments with potted plants of tomato, cabbage, pepper, eggplant, and soybean, the rate of water loss was increased after the application of Bordeaux although this increase was more pronounced for the cut leaves in potometers. The effect was greatest during the first 2-hour period following application, and showed a variation in the amount of effect with the species.

SHIVE and MARTIN (**10**), using standardized cobalt chloride paper, studied the effect of Bordeaux on the daytime transpiration rate of tomato plants in the field and found that there was a decided increase after spraying. DUGGAR and BONNS (**2**), by weighing potted plants of potato, tomato, marguerite, and tobacco, obtained data which indicated that the increase in the rate of water loss resulting from the application of Bordeaux mixture occurred almost entirely during the night. Potted plants of *Cyperus esculentus* showed no increase. Leaves of castor bean in a potometer gave a continuously increased rate of transpiration, the increase not being confined to the night period. MARTIN and CLARK (**9**) experimented with potted potato plants grown in soils of three different moisture contents, and found that the greatest average increase in water loss occurred in the plants grown at the highest soil moisture content. WILSON and RUNNELS in several papers (**12** to **19**) report an increased transpiration rate of different species for plants sprayed with Bordeaux and other compounds. They also found that the greatest increase in water loss occurred at night. CHILDERS (**1**) found, on the contrary, no increase in the rate of water loss from potted tomato plants sprayed with Bordeaux. KRAUSCHE and GILBERT (**7**), in a study of tomato plants treated with copper sprays, reported an increase in the transpiration rate, especially at night. They could find no visible change in the stomata after spraying.[2]

Recently some experimentation has also been done on the effects of oil sprays on the transpiration of sprayed plants, from which data it is apparent

[2] See also HORSFALL, JAMES G., and HARRISON, A. L. Effect of Bordeaux mixture and its various elements on transpiration. Jour. Agr. Res. 58: 423–443. 1939, for additional data published since the completion of this paper.

that the application of oils brings about a reduction in the rate of water loss (**5, 6**). Very little work has been done on calcium arsenate in this connection.

Methods

Two different methods of determining the rate of water loss before and after spraying were used. For both of these methods, the test plants were grown in the same way and used when at the same stage of development. The cranberry bean seeds were selected for uniformity in size, the average sized seeds being chosen; the large or small beans were discarded. They were soaked for six hours, drained, allowed to germinate in a moist atmosphere, and then planted in sand in paraffined trays watered with a cotton wick dipping into an attached water jar (**11**). All of the plants to be used indoors were kept in a culture room until used, while those to be used in the greenhouse were moved into it when the hypocotyls were about ten centimeters long. By indoors is meant a basement culture room kept at a constant temperature, 24° to 26° C., and a constant relative humidity of 50 per cent. Light was supplied by several 100-watt lights for 19 hours each day. The room was aerated with fans, but free from drafts, being divided into compartments which deflected the air currents. This room was an ideal place for experimentation with potometers. The plants were used for experimental purposes as soon as the first two leaves were fully expanded. The terminal bud was removed to prevent further growth of the plants, since they became very weak and spindly under the artificial light. In order to keep the experimental conditions uniform, this procedure was followed with the greenhouse plants as well.

Potometer determinations

While doubts may be raised as to the quantitative value of data derived from studies of water absorption by cut plants in potometers, the fact remains that this method affords a means of determining rapid changes in the rate of water intake. With mature plants over short periods of time these changes in the rate of absorption should reflect rather accurately the rate of water loss from the leaves. That the transpiration rate of cut shoots differs quantitatively from that of intact plants cannot be denied, but experiments on different samples under the same environmental conditions should yield comparative data of value.

Four potometers were arranged under a light in a draft-free compartment in the constant temperature culture room. The bean stems bearing two well expanded unifoliate leaves were cut under water, inserted into holes in a rubber stopper which was quickly sealed into the potometer; two stems were used for each potometer. The different samples were allowed to stand for an hour. At the end of this period readings were made every

five (or in some cases, ten) minutes for an hour; the test materials were then applied and as soon as the leaves were dry, readings were again made for several hours. A duplicate series was run the next day, using fresh plants and changing the order of the potometers to obviate any effects of variations in the tubes or differences in the light conditions. Some tests with an atmometer, substituted for the bean shoots, showed that fluctuations caused by slight changes in the environmental factors were negligible. The amount of water loss from the atmometer was quite constant, showing none of the fluctuations characteristic of the curves which show the water absorption by bean stems.

Preliminary experimentation showed that variation in the method of applying the spray materials made no important differences in the order of the results obtained. A standard procedure was adopted which consisted of wetting the under surfaces of all of the leaves with distilled water from an atomizer, dusting the wetted areas with the materials to be tested, and then rewetting the dusted surfaces until droplets of a suspension formed. This method of application brought about the least injury with unsafe materials and eliminated any possible differences due to light effects on the whitened surfaces. In all of the experiments, one potometer contained check plants sprayed with water only, one contained plants treated with ordinary commercial calcium arsenate, and the two remaining were used to test various other materials. The materials tested were: zinc-safened, basic, and ordinary commercial calcium arsenates, lead arsenate, a solution of dicalcium arsenate, Bordeaux, copper sulphate, lime, and Bancroft clay. The ordinary commercial calcium arsenate gave a test for 5 per cent. water soluble arsenic when 2 grams in a liter of water were in equilibrium with the carbon dioxide of the air, and caused 100 per cent. injury on bean leaves kept wet for 2 hours after spraying. The basic calcium arsenate under the same conditions yielded 2.5 per cent. water soluble arsenic and brought about only moderate injury on sprayed bean leaves kept wet for two days, while the zinc-safened material showed 0.7 per cent. water soluble arsenic, and caused only slight injury after the sprayed leaves had been kept wet for two days after spraying.

Two checks on the changes in the rate of water absorption were possible. One was the rate of absorption of all of the plants before treatment with the test materials, the other was the rate of absorption of the check plants throughout the experiment. The importance of carrying out potometer experiments under as constant conditions as possible is quite apparent. For determinations over short periods of time, such as 5- or 10-minute intervals, air currents can cause a considerable error, which may not become apparent if the check samples are not touched by the moving air. Hence for such measurements, the environment outdoors, or even in the green-

house, was too variable from moment to moment to allow accurate readings to be made.

Experiments with potted plants

In order to obtain data showing the effects of spray materials over a longer period of time, some experiments were set up using bean plants grown in sand in paraffined trays fitted with bottles which furnished a constant water supply to the sand through a cotton wick (**11**). The water in the bottles was replenished as often as the level became low. At times there was a change of five centimeters in the water level. The roots appeared

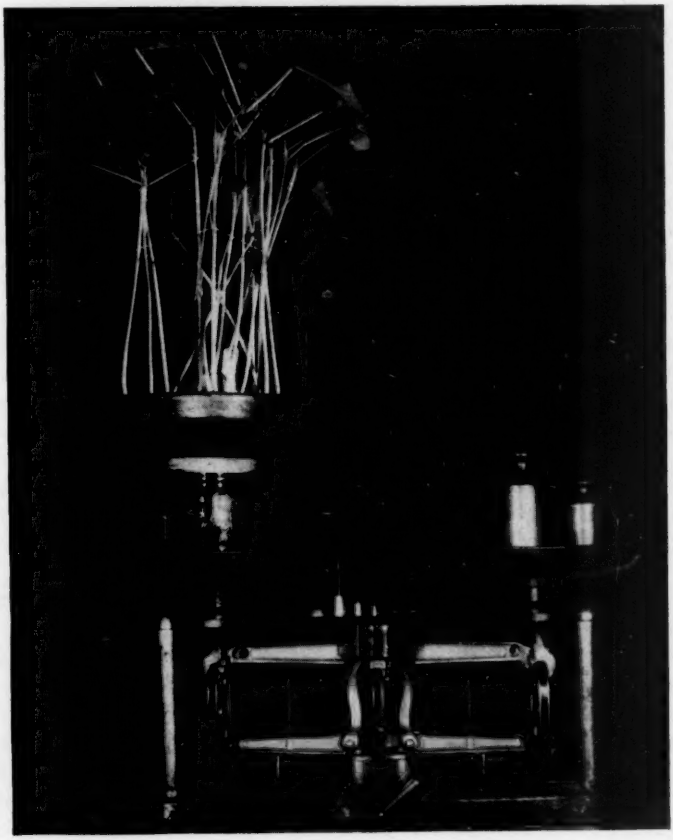

Fig. 1. Photograph of one of the trays of bean plants used in the weighing experiments to determine the amount of water lost by the plants.

healthy and apparently were not injured by the rather poor aeration of the sand. In the indoor culture room, the plants remained in good condition for from 2 to 3 weeks when the terminal bud was removed regularly. Figure 1 shows the tray with the attached water supply as the samples were weighed to determine the amount of water loss.

When the plants had fully matured, unifoliate leaves, the stand was thinned to 14 plants per tray, the sand covered with a paraffin-vaseline sealing compound, and the various trays arranged indoors or in the greenhouse. A control, set up in the same way but without plants, lost no weight over a period of several days. Two or three trays were used for each material tested, and, in addition, the experiment was repeated at least twice, each time with slight variations in procedure but showing no significant differences in the order of the results. The trays, including the jars of water, were weighed twice daily just after dark at night, and just before daylight in the morning. In the winter months, this was about 6:00 A.M. and 6:00 P.M. These data gave an indication of the effects of periods of light and darkness upon the transpiration rate of the greenhouse plants. A duplicate series was also run indoors and weighed at the same time; the plants had a longer light period indoors since the lights were on 19 hours a day.

The plants were weighed morning and evening for three or four days, before treatment with the spray materials, in order to get the relative rates of the various samples. The different trays were selected so that the samples used for each compound were assorted with one tray of plants showing a high transpiration rate, one an average rate, and one a low rate. An attempt was made to distribute the different samples at random, and to arrange them so that one of each set was surrounded by plants and the other two were at opposite edges of the table. A comparison of the rates of water loss from samples at different positions on the table did not reveal any correlation between the amount of transpiration and the position of the tray. As a precautionary measure, however, the random distribution was used.

After treatment with the test materials, the plants were weighed for ten days or two weeks afterwards depending upon the condition of the plants. There was some increase in leaf areas of the unifoliate leaves under greenhouse conditions but it appeared to be approximately equal for all of the samples. In one series, the terminal bud was allowed to grow, with the result that the plants differed considerably in amount of growth; the ones sprayed with zinc-safened calcium arsenate showed the greatest growth and made such a difference in leaf areas that the transpiration rates of the different samples could not be compared. A certain amount of injury with the ordinary commercial calcium arsenate was unavoidable, but was kept at 5 per cent. or less. The materials were applied as dusts to the wetted under-

surfaces of the leaves as in the potometer experiments. The compounds tested were: zinc-safened, basic, and ordinary commercial calcium arsenates, a solution of dicalcium arsenate, copper sulphate, lime, silica,[3] and Bancroft clay.

Some tests using cobalt chloride paper on bean plants in the field were set up to determine the time when the night changes in water loss occurred. The data, however, were difficult to interpret and yielded no significant

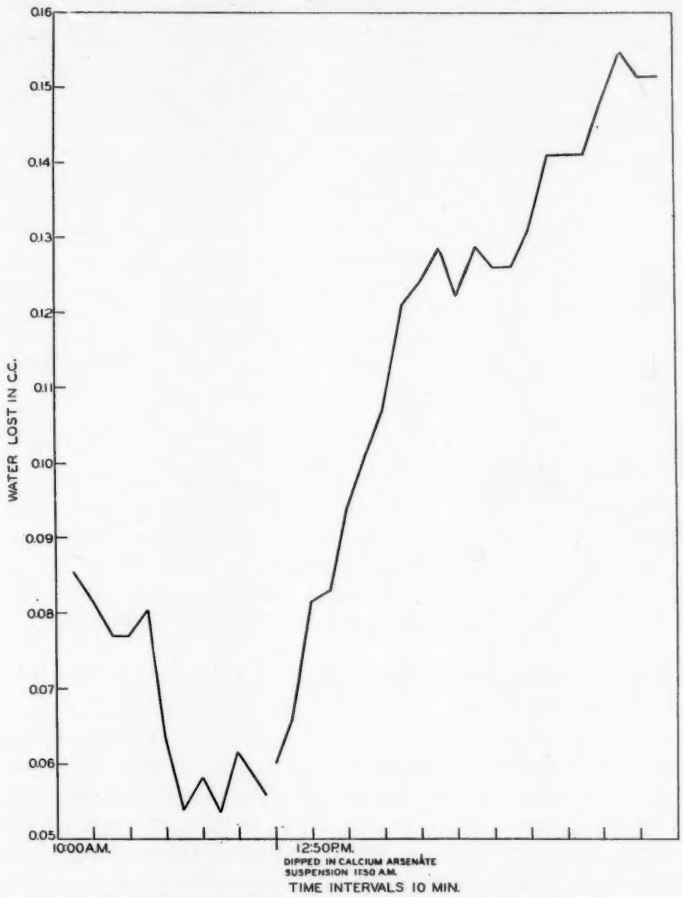

FIG. 2. Potometer curve of the rate of water absorption by bean shoots before and after treatment with ordinary commercial calcium arsenate.

[3] The words silica and silicon dioxide are used interchangeably, both referring to the pure compound silicon dioxide in powdered form.

information. Several errors were possible. One was the effect on the cobalt chloride paper of the material dusted on the leaves, and another was the error introduced by the presence of condensed moisture on the leaves at night. In addition, the color change was difficult to determine with artificial light. Since the data from the other methods showed such uniformity of results, it was felt that this method was in error rather than that the other data were invalidated.

Results

Figure 2 shows the immediate increase in the rate of water absorption of the bean shoots after treatment with ordinary commercial calcium arsenate. This result was characteristic of all the materials tested. Whether the effect was to increase water absorption or to decrease it (as in the case of copper sulphate), the change in rate was apparent as soon as the leaves had dried. The results shown in figure 3 indicate that the zinc-safened

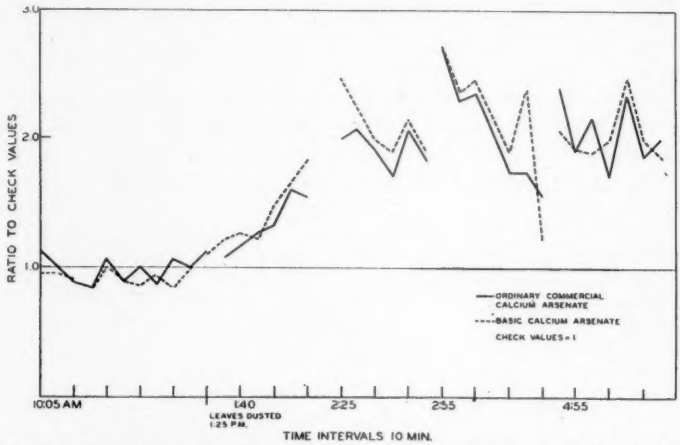

FIG. 3. Potometer curve of the rate of water absorption by bean shoots before and after treatment with ordinary commercial and basic calcium arsenates.

calcium arsenate brought about nearly the same amount of increase in water absorption as did the ordinary commercial material when both are compared with controls sprayed with water. For the preceding graph and for those that follow, the check values were reduced to unity in order to reduce the number of graphs per figure and to show the results more clearly. For these calculations, the average number of grams of water lost by the check samples was divided into the average number of grams of water lost by the samples treated with any one material for that same period of time. In this way a correction was made for weather changes such as

rain following sunshine, as well as for the physiological changes taking place in the plants with the passage of time. The check value, therefore, became a straight line. The deviations from the line by the other curves should indicate the relative amount of change brought about by the application of the test materials.

Other materials which brought about increases in the rate of water absorption as measured by potometers were lead arsenate, Bordeaux, hydrated lime, and Bancroft clay. The application of copper sulphate under the same conditions resulted in a decrease in the rate. During the course of

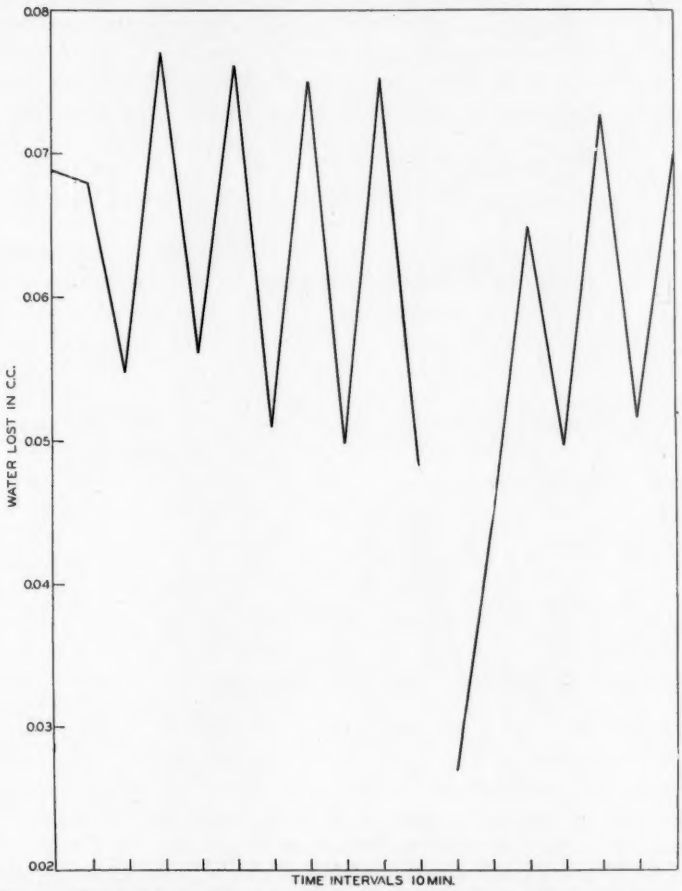

FIG. 4. Potometer curve showing rhythm in the rate of water absorption by two shoots of the check plants.

experimentation with cut plants in potometers, some curves of water absorption were obtained which showed unusual rhythmic fluctuations (fig. 4). Tests with an atmometer in place of the bean stems showed that these variations were not a result of changing environmental conditions, such as air currents, nor of physical forces involved in the movement of a bubble down the tube, for the atmometer curves showed no such variations. Apparently

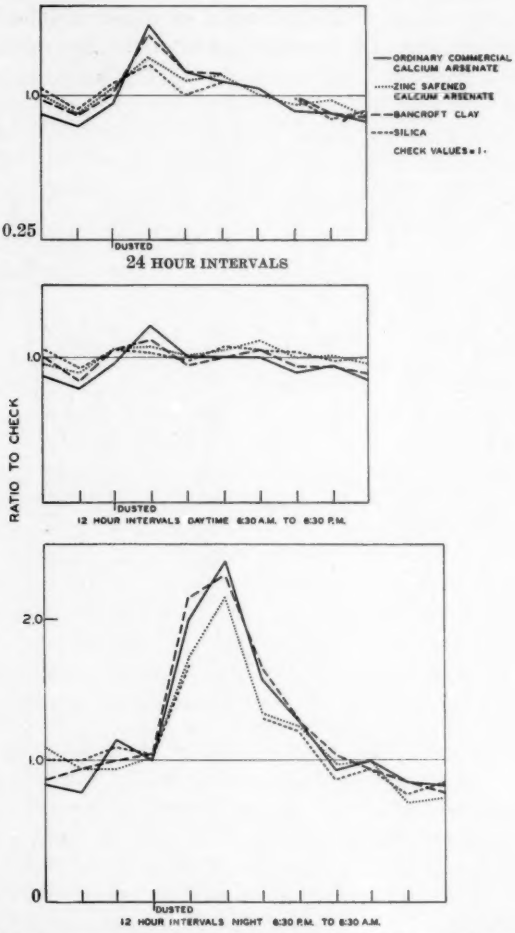

FIG. 5. Relative amounts of water lost from check plants and plants treated with certain materials applied as dusts to the wetted leaves. The test samples were potted bean plants in the greenhouse.

the fluctuations were brought about by physiological changes within the plants,—perhaps a lag of water absorption by the stem behind the water loss from the leaves. Sometimes the data from two samples run on the same day would show these variations, with one curve showing maximum where the other showed minimum.

Figure 5 and table I show the comparative effects of the zinc-safened and ordinary calcium arsenates, Bancroft clay, and silica, on the amount of water loss from potted bean plants in the greenhouse. The table is included to show the absolute amount of water lost by the different samples. The amounts of loss for the 24-hr. period and the 12-hr. periods of daylight and darkness show that the greater change occurs during the night period. These results are in accord with those of DUGGAR and COOLEY (**4**), and WILSON and RUNNELS (**12**). The total amount of water lost by the treated plants for the 24-hr. period was not much greater than that lost from the check plants (for the greenhouse samples), which fact may account for some of the discrepancies in the literature. The 24-hr. measurements might not reveal the great increase in transpiration of the sprayed plants at night as compared with the checks, since the total number of grams of water lost by any of the samples at night is usually so much less than the amount lost during the daytime that the changes might not be apparent.

The curves also show that such materials as silica and Bancroft clay can bring about increases as great as those resulting from the application of ordinary spray materials. To ascertain whether or not these results might be attributed to some chemical contamination of the silica and Bancroft clay, another series was run, using these materials washed until chemical tests showed scarcely a trace of any other materials present. The results were the same for the second series. After several days the rates of all of the treated plants began to approach the original rates of water loss.

The curves (fig. 6) and data in table II show the effects of the same materials tested on a similar series of plants kept indoors in the culture room. The high rate at one time before treatment is apparently caused by an error in recording the loss in weight of the check plants. Here there is no pronounced difference between the daytime and the night losses. These plants had only 5 hours of darkness, being illuminated for 19 hours every day with electric lights. These curves show a separation into pairs, the ordinary commercial calcium arsenate and the Bancroft clay bringing about approximately the same increases, and the zinc-safened calcium arsenate and the silica showing somewhat similar effects. Here the application of a zinc-safened material did not bring about as great an increase in the transpiration rate as did the ordinary commercial calcium arsenate. The effect of the latter, however, may be somewhat exaggerated, because the plants in these samples showed as high as 10 or 20 per cent. injury. What the water

TABLE I

LOSS IN WEIGHT OF POTTED BEAN PLANTS IN THE GREENHOUSE BEFORE AND AFTER TREATMENT WITH CERTAIN MATERIALS*

SAMPLE	JAN. 7	JAN. 8	JAN. 9	JAN. 10	JAN. 11†	JAN. 12	JAN. 13	JAN. 14	JAN. 15	JAN. 16	JAN. 17	JAN. 18	JAN. 19	JAN. 20
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
WATER LOSS IN 24 HOURS (AVERAGE OF TWO TRAYS)														
Ordinary commercial calcium arsenate	.....	65	80	58	.....	99	91	92	98	84	105	93	79	104
Zinc-safened calcium arsenate	.....	66	83	58	.....	79	82	84	95	81	102	87	72	83
Bancroft clay	.....	76	90	63	.....	86	87	94	104	88	102	93	83	101
Silicon dioxide	.....	78	92	65	.....	82	86	89	97	90	101	95	83	80
Check	.....	72	101	61	.....	66	79	83	91	88	110	102	86	90
WATER LOSS DURING THE PERIOD 6:45 A.M. TO 6:45 P.M.														
Ordinary commercial calcium arsenate	.....	54	70	48	.....	55	74	69	79	63	65	67	53	61
Zinc-safened calcium arsenate	.....	56	72	48	.....	46	68	65	78	62	65	67	50	58
Bancroft clay	.....	62	78	53	.....	43	71	70	81	66	65	70	59	58
Silicon dioxide	.....	62	81	54	.....	48	73	72	80	71	65	72	58	61
Check	.....	61	88	50	.....	46	72	68	76	66	66	73	56	57
WATER LOSS DURING THE PERIOD 6:45 P.M. TO 6:45 A.M.														
Ordinary commercial calcium arsenate	11	10	10	10	44	17	23	19	21	41	26	26	43	46
Zinc-safened calcium arsenate	10	11	10	10	33	14	19	17	19	37	20	22	25	29
Bancroft clay	14	12	10	11	43	16	24	23	22	37	23	24	43	41
Silicon dioxide	16	11	11	15	34	13	19	17	19	36	23	25	19	53
Check	11	13	11	10	20	7	15	15	22	44	29	30	33	50

* Blank spaces indicate that the weighings were omitted at the beginning of the experiment and during the day on which the plants were treated with the test materials.

† The plants were treated with the test materials during the afternoon.

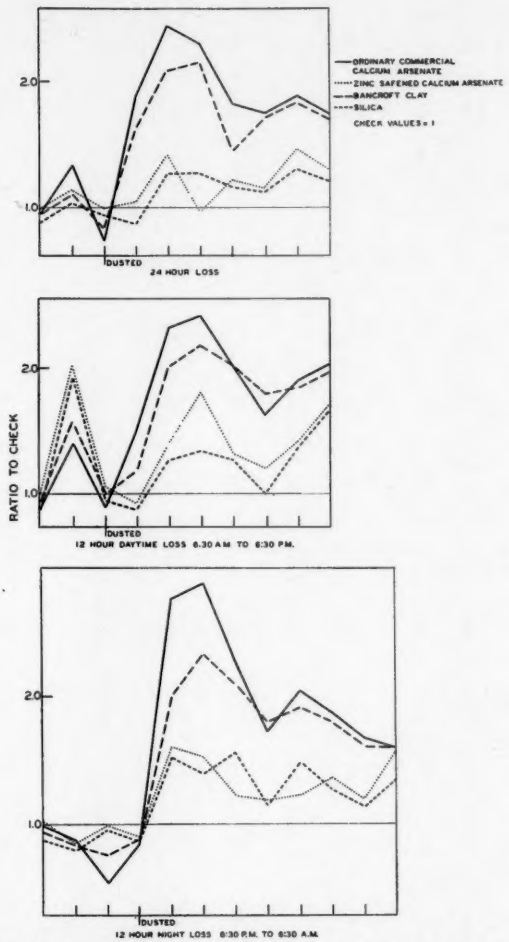

FIG. 6. Relative amounts of water lost from check plants and plants treated with certain materials applied as dusts to the wetted leaves. The test samples were potted bean plants in the indoor culture room.

loss from such injured areas may be has not been determined. For these plants the increased rates of water loss do not tend to return to the original rates.

A comparison was then made of the effects of washed Bancroft clay and silica suspended in water and in a solution of dicalcium arsenate containing one-half per cent. water-soluble arsenic. The leaves were dipped

TABLE II

LOSS IN WEIGHT OF POTTED BEAN PLANTS IN THE INDOOR CULTURE ROOM BEFORE AND AFTER TREATMENT WITH CERTAIN MATERIALS*

SAMPLE	JAN. 7	JAN. 8	JAN. 9	JAN. 10	JAN. 11†	JAN. 12	JAN. 13	JAN. 14	JAN. 15	JAN. 16	JAN. 17	JAN. 18	JAN. 19	JAN. 20
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
WATER LOSS IN 24 HOURS (AVERAGE OF TWO TRAYS)														
Ordinary commercial calcium arsenate	.....	21	22	13	.....	47	44	39	34	31	30	29	28	35
Zinc-safened calcium arsenate	.....	24	19	17	.....	29	26	20	22	19	21	25	23	18
Bancroft clay	.....	28	27	14	.....	46	38	37	35	31	29	31	34	36
Silicon dioxide	.....	26	17	20	.....	28	23	21	21	20	21	17	24	24
Check	.....	22	21	17	.....	25	18	17	18	18	17	19	24	21
WATER LOSS DUIRNG THE PERIOD 6: 45 A.M. TO 6: 45 P.M.														
Ordinary commercial calcium arsenate	.....	12	13	8	.....	22	22	20	18	15	14	15	14	17
Zinc-safened calcium arsenate	.....	12	10	8	.....	14	13	10	11	10	10	12	10	10
Bancroft clay	.....	12	15	7	.....	28	19	19	18	16	14	16	18	22
Silicon dioxide	.....	11	9	12	.....	14	12	11	11	9	10	7	12	13
Check	.....	12	11	8	.....	16	10	9	9	10	8	9	14	11
WATER LOSS DURING THE PERIOD 6: 45 P.M. TO 6: 45 A.M.														
Ordinary commercial calcium arsenate	9	9	5	7	25	22	19	16	16	16	14	14	18	14
Zinc-safened calcium arsenate	12	9	9	8	15	13	10	10	9	11	10	13	13	10
Bancroft clay	16	12	7	8	18	19	18	17	15	15	15	16	14	18
Silicon dioxide	15	8	8	11	14	11	10	10	11	11	10	12	11	12
Check	10	10	9	8	9	8	8	9	8	9	10	10	10	11

* The blank spaces indicate that weighings were omitted at the beginning of the experiment and during the day on which the plants were treated with the test materials.

† Treated with test materials during the afternoon.

in a 2–100 suspension. The silica was further separated into coarse and fine particles by allowing it to settle in water. An attempt was made to separate out the particles smaller than the stomatal aperture of the bean leaf from those larger. It is apparent from the curves shown in figure 7

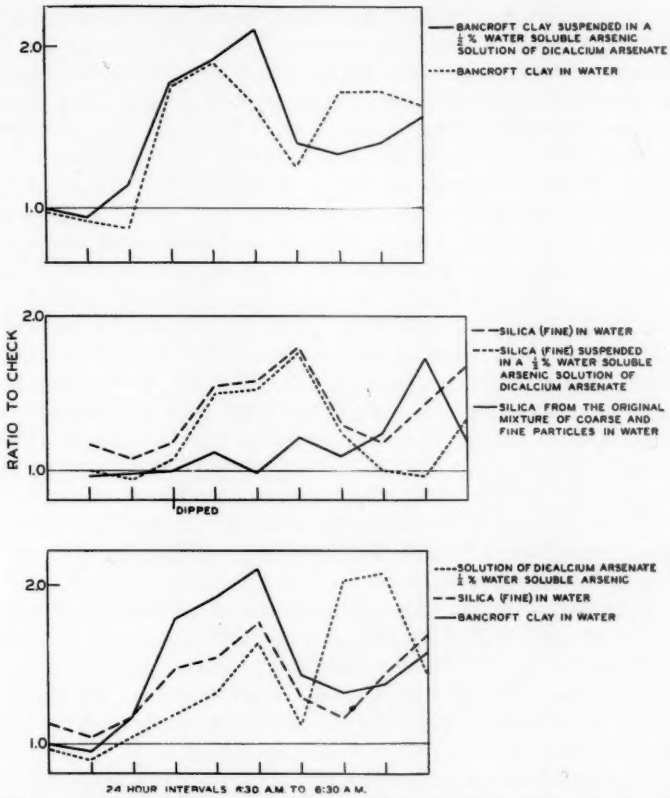
2.0
1.0
BANCROFT CLAY SUSPENDED IN A ½ % WATER SOLUBLE ARSENIC SOLUTION OF DICALCIUM ARSENATE
BANCROFT CLAY IN WATER
RATIO TO CHECK
SILICA (FINE) IN WATER
SILICA (FINE) SUSPENDED IN A ½ % WATER SOLUBLE ARSENIC SOLUTION OF DICALCIUM ARSENATE
SILICA FROM THE ORIGINAL MIXTURE OF COARSE AND FINE PARTICLES IN WATER
DIPPED
SOLUTION OF DICALCIUM ARSENATE ½ % WATER SOLUBLE ARSENIC
SILICA (FINE) IN WATER
BANCROFT CLAY IN WATER
24 HOUR INTERVALS 6:30 A.M. TO 6:30 A.M.

FIG. 7. Relative amounts of water lost from check plants and plants dipped in suspensions of the test materials. The samples were potted bean plants in the indoor culture room. Check value = 1.

that particle size affects the amount of change in the rate of water loss. It is also obvious that chemically inert materials can bring about quite as large increases in the transpiration rate as physiologically active materials such as dicalcium arsenate.

Discussion

From the above results, several interesting conclusions may be drawn. In the first place, it is apparent that the relative safety of one calcium

arsenate over another is not correlated with its effect on water loss. Second, chemically inert and innocuous materials such as Bancroft clay and silica, as well as such a physically inert material as soluble dicalcium arsenate, can both bring about very significant increases in the transpiration rate. Particle size is a factor affecting the amount of increase. Third, for greenhouse plants, the greatest relative increase in water loss of the treated plants occurs at night.

The question then arises as to the nature of the action of these materials which bring about such changes in the transpiration rate. Very few explanations have been offered in the literature. Early workers suggested the effects of the white coating on light absorption, leaf temperatures, or photosynthesis. These factors could not have entered into the problem here because the test materials, with one exception, were always applied only to the undersurfaces of the leaves. DUGGAR and BONNS (2) suggested the influence of surface films on water loss from the stomata and hydathodes since they also found a decrease in the effect with time. KRAUSCHE and GILBERT (7) thought there must be an effect on the cuticular transpiration.

It is difficult to see what changes in cuticular transpiration could take place after the application of chemically inert materials in the form of dusts. It is hardly possible that compounds like chemically pure silicon dioxide could change the permeability of the epidermal cells to such an extent that more water could pass through the walls, especially when they are applied in such a manner as to prevent injury to the leaf. Since differences in particle size brought about significant differences in the amount of change in the rate of water loss, it appears that perhaps an effect on the stomata might explain the facts more easily.

It is significant that the greatest difference in the amount of water loss between the treated plants and the check plants in the greenhouse should occur at night. Since cuticular transpiration presumably continues at all times during the 24-hr. period, and is not influenced by the presence or absence of light as is stomatal transpiration, any differences in this rate should be apparent in the daytime as well as the night. It should also show up as a greater increase for the 24-hr. period as well. From these data, this is not the case. The daytime, and even at times the 24-hr. increase in rate, is almost negligible for the greenhouse plants. Even though the amounts of water lost at night are small, it seems that there should be more of a difference than there is in the daytime rate since the increases would be cumulative for the entire 24 hours. On the other hand, if one postulates that the effect is on stomatal transpiration, it is easy to account for the fact that the increase is always greater at night and almost negligible during the day, when the stomata would be open anyway.

Examination of the dusted leaves with a microscope showed particles

partially blocking the stomata. This observation, coupled with the fact that the smaller sized particles brought about a greater increase in the transpiration rate, indicates that the blocking of stomatal openings by these particles might prevent complete closure of the stomata. Since not all of the stomata would be blocked, necessarily, the rate of water loss would not be expected to approach the daytime rate; it would, however, be greater than the cuticular loss alone. That the greenhouse plants outgrow, so to speak, the effect of these materials, seems to be a result of an increase in area of the sprayed leaves, maturation of undeveloped stomata, and a gradual loss of the spray particles. Thus transpiring areas unaffected by previous treatment are acquired by the plant.

When indoor plants were used in the same experiment, there was neither the great change in the night rate of transpiration, nor the gradual return of the increased rates to the original values. The first effect appears to be related to the day-night rhythm of transpiration effective in the greenhouse, and the second to the increase in area of the sprayed leaves which does not take place indoors under artificial light. Under these conditions, the accelerating effect of the inert materials (Bancroft clay and silica) upon the rate of water loss is still more difficult to explain, even when taken into consideration as effect on the stomata. Since there are no data available on the condition of the stomata in plants grown under these conditions, and one does not know whether or not the artificial light can affect the opening and closing of the stomatal apertures, it is impossible to speculate as to what the effect may be until more data are obtained. As in the case of the greenhouse plants, it is difficult to account for the effect of chemically inert materials on the amount of water passing through the cuticle.

That there is a chemical, as well as a mechanical effect, is evidenced by the fact that a solution of dicalcium arsenate will also bring about increases in the rate of water loss of the treated plants. There are two possible explanations of this result. Either there is a chemical effect on the cell walls, rendering them more permeable and thus increasing cuticular transpiration, or there is an effect on the guard cells of the stomata which causes the stomata to remain open longer. It is known that the guard cells differ from the other epidermal cells in physiological activity. The fact that certain workers have reported a greater increase in transpiration when the leaves were sprayed on the lower surfaces as compared with leaves sprayed on the upper surfaces, also indicates that the action of these chemically active materials may be on the stomatal responses rather than on cuticular permeability, since the plants studied had more stomata on the lower epidermis than on the upper. An investigation into the condition of the stomata of the sprayed and unsprayed plants at all times of the night and day will be necessary before an explanation of these effects of sprays and other materials can be based on direct evidence rather than inferred from experimental data.

Summary

Zinc-safened, basic, and ordinary commercial calcium arsenates, chemically pure Bancroft clay and silica, and a solution of dicalcium arsenate all brought about significant increases in the transpiration rate of bean plants, as determined by both a study of cut shoots in potometers and the loss in weight of potted plants.

The application of zinc-safened and basic calcium arsenates brought about as great an increase in the rate of water loss of the treated plants as did the ordinary commercial material. In this case the phytocidal action of a compound is not correlated with its capacity to increase the transpiration rate of the injured plants. If injury becomes apparent, however, as burned areas on the leaves, these plants will show a higher rate of water loss than the uninjured plants.

For greenhouse plants, the increased rate of water loss was most apparent at night when the treated plants were compared with controls. This was not the case for the plants kept in the indoor culture room.

Chemically inert Bancroft clay and silica, as well as physically inert dicalcium arsenate in solution, brought about equally large increases in the transpiration rate of the treated plants as compared with controls.

An attempt is made to explain some of the results on the basis of changes in stomatal action brought about by the application of the different materials studied.

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POLYSACCHARIDES OF THE VEGETATIVE TISSUES OF MAIZE[1]

C. G. Barr

(WITH ELEVEN FIGURES)

Introduction

The polysaccharides of the vegetative tissues of maize have been designated as starch or starch-like by a number of investigators (**14, 16**) who have considered acid hydrolyzable materials to be starch. Colin and de Cugnac (**2**), however, found that sugars, dextrins, and levulins are the characteristic reserves of the vegetative plants of the Gramineae, even of the cereals in which starch is the reserve polysaccharide of the fruit. Loomis (**11**) also found that polysaccharides do not constitute an important fraction of the vegetative maize plant, even under conditions favorable to maximum accumulation of reserves within the plant. The study of the polysaccharides of maize involves, then, the more exact determination of the compounds present, as well as their relation to photosynthesis, translocation, and growth within the plant.

Materials and methods

The material used was obtained from a selection of open pollinated Reid's Yellow Dent maize. Samples were taken at 3-hr. intervals beginning at 4 A. M. and continuing for 24 hours, unless otherwise indicated. From 15 to 20 stalks were selected at random from a row of 60 hills at each sampling. The middle portion of each stalk bearing two leaves was brought quickly to the laboratory, where the leaves were removed and distributed into two groups so that an upper leaf from each alternate stalk was contained in each group. After the leaves were split down the midrib, duplicate 100-gm. samples of the blades were weighed to $\pm$ 0.10 gm. Each sample was then cut over and allowed to fall into a quart Mason fruit jar containing 450 ml. of boiling 95 per cent. alcohol. The quantities of alcohol were such as to give a final concentration of 80 per cent. when diluted by the water in the samples. After simmering for about 30 min. the jars were sealed and stored.

Duplicate samples of the stalk sections from which the leaves had been removed were taken also. The section of the stalk used for sampling was obtained from the first internode above the ear, or as near this point as possible in the first samplings, which were made before the ear shoot showed

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above the leaf sheath. The ends of the internode were cut away and the central portion of pith and rind quartered longitudinally. One-hundred-gram samples were cut into 2- to 4-mm. pieces and allowed to fall into jars containing 450 ml. of boiling 95 per cent. alcohol. These were boiled gently on a steam bath for 30 to 40 min., sealed, and stored.

Soluble carbohydrates were extracted with 80 per cent. alcohol before the polysaccharide analyses were begun. The residues from the 80 per cent. alcoholic extraction were dried to constant weight under reduced pressure at 65° C. The dried residues were ground in a Wiley and a ball mill until 98 per cent. or more of the sample would pass through a 200-mesh sieve.

The polysaccharides were extracted from this ground residue by various methods. Normally the first extraction was made with cold 10 per cent. alcohol (9), the second with hot water, the third with saliva, and the fourth with boling 1+20[2] HCl. The first three extracts were cleared, made to volume, filtered, deleaded, aliquots hydrolyzed with HCl (1+20), and the reducing value of the extract determined. Clearing was omitted for the acid-hydrolyzable extract which was cooled, neutralized, made to volume and tested for reducing substances. Clearing was also omitted from some of the water extracts, or aliquots of these extracts, to determine the reducing value of the clearing precipitate.

Hydrolysis of the extracts was accomplished by refluxing, sometimes on an open flame turned low and sometimes in a boiling water bath, or by autoclaving with 1+20 or 1+40 HCl at 120° C. When the method of hydrolysis is not indicated, 1+20 HCl at 120° C. for one hour was used. The results are expressed in the main as percentage glucose equivalent on the basis of green weight. In the extraction and hydrolysis experiments, the results are expressed as milligrams of glucose equivalent per 50 ml. of uniform aliquots.

Experimental results

Fractionation of the polysaccharides of maize

In the absence of published methods for the fractionation of the polysaccharides of maize it was necessary to perform a number of experiments for the development of suitable methods. Eight 1.25-gm. samples of a composite lot of powdered, sugar-free maize leaf tissue were weighed carefully and transferred to 50-ml. centrifuge tubes. Thirty to 40 ml. of cold 10 per cent. alcohol was then added and allowed to stand 20 min. with occasional stirring. The samples were divided into lots of 4 each and carried through in a duplicate series of extractions. Two successive extractions from the 4 tubes were combined in 250-ml. volumetric flasks for each

[2] One part concentrated HCl and 20 parts extract by volume.

determination. Aliquots of the extracts were cleared and prepared for hydrolysis as described above. Fifty-ml. portions of the cleared and uncleared solutions were then tested for reducing substances which were calculated as mg. of dextrose per 50-ml. aliquot. The results of the tests on the different extractions are shown in table I. A heavy yield of reducing substances was obtained in the combined first and second extractions. The third and fourth extractions dropped to a low yield, which continued with slight decrease and some fluctuation through the eighteenth extraction. The first four extractions were considered to have removed a distinct polysaccharide fraction which was designated as the alpha fraction.

The residue in the centrifuge tubes after the cold 10 per cent. alcohol extractions was covered with 30 to 40 ml. of boiling distilled water, the rack containing the tubes was placed in a boiling water bath for 20 min. and the contents stirred at intervals. Successive extractions were combined, aliquots cleared, and the cleared and uncleared solutions tested for reducing substances. A second fraction yielding reducing substances on acid hydrolysis was removed by the hot water extraction. Normally this fraction, designated as the beta fraction, was smaller than the first although in drier seasons the beta fraction was of equal or major importance.

TABLE I

EXTRACTION OF ALPHA AND BETA POLYSACCHARIDE FRACTIONS FROM MAIZE LEAF POWDER

EXTRACTIONS	ALPHA FRACTION			BETA FRACTION		
	GLUCOSE VALUES*	GLUCOSE VALUES* TOTALS		GLUCOSE VALUES*	GLUCOSE VALUES* TOTALS	
	CLEARED	CLEARED	UNCLEARED	CLEARED	CLEARED	UNCLEARED
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1 and 2	27.30	27.30	33.50	6.40	6.40	9.60
3 and 4	0.43	27.73	35.45	1.70	8.10	12.20
5 and 6	0.40	28.13	36.70	0.70	8.80	13.35
7 and 8	0.20	28.33	37.95	1.15	9.95	14.45
9 and 10	0.00	28.33	38.15	0.20	10.15	14.80

* Mg. glucose equivalent per 50 ml. of uniform aliquots.

The glucose equivalent values of the first 10 extractions for alpha and beta polysaccharides are shown in table I. As already indicated, continued extractions gave fluctuating low values suggesting slow hydrolysis of the substrate. The beta fraction was not only insoluble in cold 10 per cent. alcohol, but was rather slowly extracted by boiling water. The first 2 cold extractions removed 96 per cent. of the material obtained in 10 extractions, whereas the first two hot extractions removed only 63 per cent. of the total. Other differences in the two fractions are discussed later.

The beta polysaccharide fraction gave a brown color with I–KI solution,

even when concentrated by precipitating from solution with alcohol, and was in consequence considered to be starch-free. The residue from the 10 per cent. alcohol and hot water extractions was gelatinized, treated with tested saliva, and analyzed for starch by the method given by LOOMIS and SHULL (13). The reducing sugar yields were not greater than were obtained by hot water extraction alone. Microchemical tests for starch on fresh and preserved maize tissue at various mid- and late-season stages have failed uniformly to give positive reactions although scattered starch grains have been observed in the base of young plants. Fresh leaves from defruited plants have on occasion shown a faint lavender color of amylodextrin, but other materials have stained brown with iodine. Our results modify the conclusions of COLIN and DE CUGNAC (2), who classify maize as storing sucrose rather than starch, to sucrose plus two dextrin-like fractions, but little or no true starch in the vegetative tissues of maize.

The reducing substances removed by boiling 1 + 20 hydrochloric acid in a standard time, usually two hours, are frequently reported as "starch." Since our tests generally showed no starch in the vegetative maize tissues at any stage of development, the term is a misnomer when applied to this plant. The use of acid hydrolysis alone is open also to the serious objection that it does not differentiate between the reserve and structural polysaccharides. In our material, where starch was absent and saliva removed no more material than did hot water, we used acid hydrolysis as a measure of the readily hydrolyzable, hemi-reserve, or more soluble structural substances, present in the dextrin-free residue. Acid hydrolysis is an empirical reaction whose yield depends upon acid strength, time, temperature, and fineness of the sample as well as upon the chemical composition of the substrate.

Data are presented in table II which show that finely ground maize leaf residues contain a large, readily hydrolyzable fraction. A point of stability

TABLE II

ACID HYDROLYSIS WITH 1 + 20 HYDROCHLORIC ACID UNDER REFLUX ON RESIDUES FROM ALPHA AND BETA POLYSACCHARIDE EXTRACTIONS

PERIOD	TOTAL TIME	GLUCOSE VALUES* FOR PERIOD	TOTAL GLUCOSE* VALUES
30 min.	30 min.	55.80	55.80
1 hr.	1.5 hr.	4.50	60.30
1.5 "	3 "	3.50	63.80
2 "	5 "	1.50	65.30
2 "	7 "	1.45	66.75
2 "	9 "	1.20	67.95
2 "	11 "	1.30	69.25
8 "	19 "	2.30	71.55
8 "	27 "	2.00	73.55

* Mg. glucose equivalent per 50 ml. of uniform aliquots.

in the hydrolysis curve was not reached, however, until the treatment had been continued for three hours. Unpublished data obtained in this laboratory by J. C. ELDREDGE show that one hour at 120° C. (autoclave at 15 pounds pressure) is equivalent to refluxing for 4 hours on a boiling water bath in the hydrolysis of maize tissues. The autoclaving method was chosen for the routine analyses because of its greater speed and convenience.

In addition to the three fractions, alpha, beta, and acid hydrolyzable, materials forming reducing substances were extracted by both hot and cold water and precipitated by neutral lead clearing. The losses from clearing shown in table I were approximately 26 per cent. of the alpha- and 31 per cent. of the beta-extraction.

PROPERTIES OF THE POLYSACCHARIDE FRACTIONS

HYDROLYSIS REACTIONS.—A 5-gm. sample of powdered leaf residue was extracted with cold 10 per cent. alcohol and the extract cleared with neutral lead acetate and filtered. Microscopic examination showed that the clearing precipitate was amorphous and of jelly-like consistency; it gave no color reaction with I–KI, and was filtered out with difficulty. Hydrolysis of the clearing precipitate with 1 + 20 hydrochloric acid at 100° C. for two hours gave slightly less than 100 per cent. recovery of the loss in reducing substances as a result of clearing. Hydrolysis at 120° C. in the autoclave, however, almost completely destroyed the reducing power of the lead-containing material.

The residue from the 10 per cent. alcohol was extracted with hot water, and the two polysaccharide extracts were used for a comparison of the effects of concentration of acid, and of time and method of hydrolysis. The maximum yield of glucose was obtained with 1 + 20 hydrochloric acid, whether hydrolyzed at 100° C. or 120° C. The difference in values by the two methods was small (fig. 1), and hydrolyzing at 120° C. had the advantage that a larger number of samples could be handled more conveniently and in considerably less time. The hydrolysis was not complete even after 4 hr. with 1 + 40 hydrochloric acid at 100° C., while longer than 2 hr. at 120° C. caused a slight destruction of reducing substances.

Hydrolysis of the alpha fraction with 1 + 20 hydrochloric acid at 100° C. for 30 min. yielded about 50 per cent. of the reducing power obtained in the same length of time at 120° C. The comparative yield for the beta fraction, when treated similarly, was considerably less, amounting to about 20 per cent.

Long continued hydrolysis with 1 + 20 hydrochloric acid at 120° C. resulted in destruction of the reducing substances from both the alpha and beta fractions. The beta fraction, however, was not as readily destroyed by the more drastic hydrolysis as was the alpha fraction. In their other characteristics the hydrolysis curves of the beta fraction were comparable to those for the alpha fraction shown in figure 1.

The method commonly used to determine the acid-hydrolyzable material in the starch- and dextrin-free residue is to reflux it for 2.5 hr. with 1 + 20 hydrochloric acid. This method does not give a distinct end point although the values obtained are comparable. Further treatment shows that more reducing substances are obtained with additional hydrolysis. In an accumulative 40-hr. hydrolysis treatment, 75 per cent. of the total reducing substances obtained was extracted in the first 90 min. of hydrolysis. It is suggested that the reducing substances produced after prolonged hydrolysis are the result of the gradual digestion of the less readily hydrolyzed materials

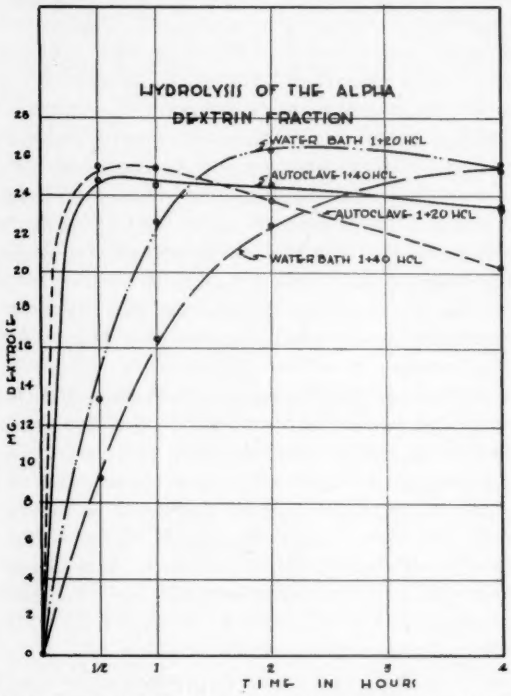


FIG. 1. Hydrolysis curves for varying treatments of alpha extract from maize leaves.

including cellulose. There was, however, a distinct line of demarcation separating the readily and more slowly hydrolyzed polysaccharide fractions, comparable to the endpoints shown in the extraction of the alpha and beta dextrin fractions. The fraction is considered to be that material removed in the first 3 or 4 extractions for the alpha and beta fractions and in the first one to two hours for the acid hydrolyzable materials. Further treatment yields more reducing substances with any of the extractions, but this yield

appears to be caused by the hydrolysis of the more complex underlying materials.

The hydrolyzed materials from each of the three methods of extraction were tested for fructose by the JACKSON and MATHEWS (**5**) modification of NYN's selective method for the estimation of levulose to determine whether levulosans had been present in the extract before hydrolysis. Negative results were obtained in all cases.

OSAZONE REACTIONS.—A composite sample of the leaf material was extracted with 10 per cent. alcohol, the extract concentrated by evaporation, cleared with neutral lead acetate, and the excess lead removed with sodium oxalate. Sufficient 95 per cent. alcohol was added to the cleared solution to precipitate the dextrin-like material of the alpha fraction. A white, amorphous substance was obtained which gave a reddish brown color with I–KI and was soluble in cold water.

Acid-hydrolyzed portions of a water solution of the precipitate yielded phenyl-osazones when treated with phenyl hydrazine hydrochloride and sodium acetate (**1**). Since dextrin is assumed to be hydrolyzed to glucose, glucosazones should have been formed. The osazones which were formed, however, crystallized out only upon cooling and more closely resembled maltosazones than glucosazones in structure.

NOYES *et al.* (**15**) have reported that glucose is not altered in reducing power by treatment with 10 per cent. hydrochloric acid. It does not follow, however, that glucose was not altered in some way since darkening of the solution after prolonged heating indicated some alteration. In order to determine whether or not the substances produced by acid hydrolysis of the precipitate might be altered by the acid treatment, and as a consequence alter the structure of the osazone formed, comparisons were made of the osazones of the alpha-dextrin fraction and c.p. dextrin.

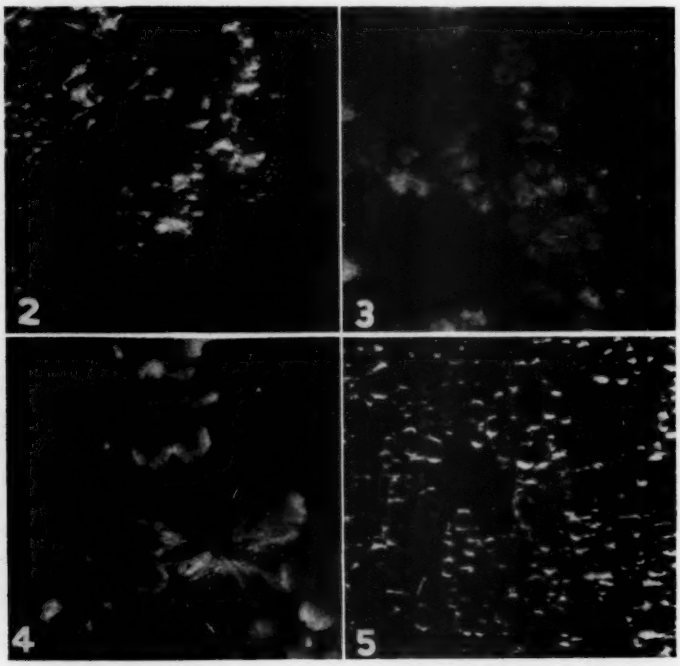
One liter of a 0.10 per cent. solution each of alpha-dextrin and of c.p. dextrin was prepared. One hundred-ml. aliquots of the two solutions were hydrolyzed with 1 + 20 HCl under varying periods of time and temperature as follows:

1. 30 minutes at 100°
2. 1 hour " "
3. 2 hours " "
4. 4 hours " "
5. 30 minutes at 120° C.
6. 1 hour " "
7. 2 hours " "
8. 4 hours " "

Figures 2 to 5 show clearly increasing degrees of alpha extract hydrolysis. The phenyl hydrazine derivatives shown in figure 2 following 30 min. hydrolysis at 100° C. were very small, star-like, crystalline bodies with many amorphous particles distributed throughout the field. Hydrolysis for one hour increased the number and produced more uniformity in the crystals. Numerous sharp yellow needles were formed although they were too small

to resemble glucosazone fragments produced by any hydrolysis treatment performed. Further hydrolysis for two and four hours at 100° C. yielded substances which formed osazones of the type shown in figure 3.

The best crystal formation was obtained with solutions hydrolyzed 30 min. at 120° C. These osazones more closely resembled those formed by glucose than any of the other treatments. Hydrolyzing longer than one hour at

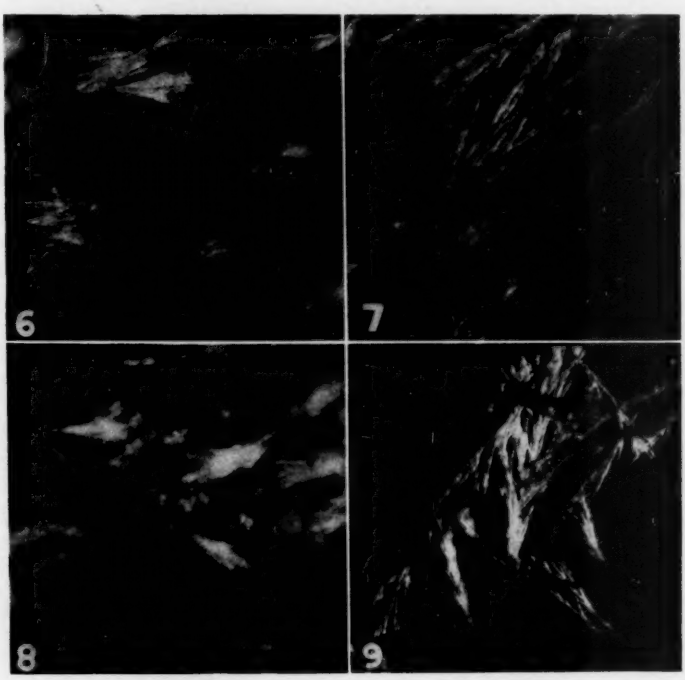


FIGS. 2–5. Phenyl osazones from alpha extract hydrolyzed in 1 + 20 HCl.
Fig. 2. Heated 30 min. at 100° C.
Fig. 3. Heated 4 hr. at 100° C.
Fig. 4. Heated 30 min. at 120° C.
Fig. 5. Heated 4 hr. at 120° C.

120° C. seemed to bring about, in this case also, a change in the products of hydrolysis and occasioned the formation of what appeared to be isomaltose.

Comparing figures 6 to 9 with figures 2 to 5 it will be observed that the glucosazones formed from dextrin were only slightly altered by the different hydrolysis treatments. It is significant that the crystals obtained from all of the dextrin solutions crystallized out in hot water after 20 to 30 min. heating, while those from the alpha extract of maize crystallized only after

cooling. This character suggests maltose or isomaltose. It does not, however, seem probable that glucose formed by gentle hydrolysis of the alpha fraction should immediately be polymerized with the formation of isomaltose, while the same treatment had no such effect upon pure dextrin. The behavior of the osazones formed by the products of hydrolysis from the alpha extract, together with their structural types show rather definitely that the material



FIGS. 6–9. Phenyl osazones from c.p. dextrine hydrolyzed in 1 + 20 HCl.
Fig. 6. Heated 30 min. at 100° C.
Fig. 7. Heated 4 hr. at 100° C.
Fig. 8. Heated 30 min. at 120° C.
Fig. 9. Heated 4 hr. at 120° C.

extracted from corn leaf tissue by 10 per cent. alcohol differs from true dextrin in unknown respects.

Although the exact nature of the substance has not been determined it appears that: (a) upon hydrolysis, reducing substances are produced which are not glucose; or (b) the presence of impure gums alters the hydrolytic products in such a fashion as to change their reaction with phenylhydrazine, masking their identity.

Phenyl osazones produced from the hot-water soluble (beta) extract closely resembled glucosazones but a mixture of osazones was observed suggesting the presence of materials other than glucosans.

Variations in the polysaccharide fractions

Diurnal variations.—Loomis (10) has reported that sucrose shows the most important diurnal variations among sugars of the leaf and stem of maize. In our experiments sucrose in the leaves increased 1.5 per cent. to four times

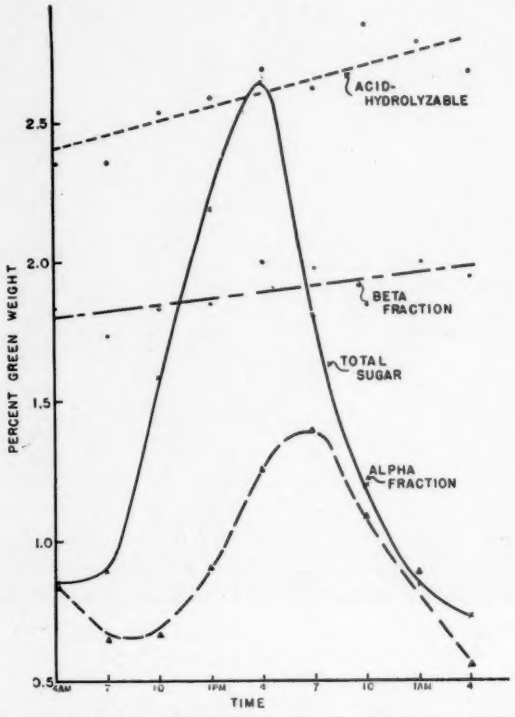


Fig. 10. Diurnal variations in the carbohydrates of maize leaves. July 19–20, 1932.

its original value between 4 A.M. and 4 P.M. Sucrose in the stem increased from a low of 0.27 per cent. at 7 A.M. to 0.90 per cent. at 7 P.M. on July 15, the maximum showing a lag of three hours in comparison with the leaf maximum. Polysaccharide analyses were made on series of samples collected July 19, 1932 and July 15, 1933. They showed variations of nearly 100 per cent. in the alpha fraction in both seasons. Neither the beta nor the acid-hydrolyzable fraction showed significant diurnal variations in the 1932 analyses. In

1933, however, when these two fractions were determined together, they showed as much variation as did the alpha fraction. Data for the two series are shown in tables III and IV and in figures 10 and 11, where the total sugar curves are included for comparison. Total sugar variations, as stated above, were mainly attributable to sucrose. Cold-water soluble polysaccharides are shown to exhibit diurnal fluctuations in the leaves of silking maize with a tendency for the maximum to follow the maximum of total sugars by about three hours. The differences in the variation of the beta fraction in the two years are not understood. In one series of ground samples left exposed to laboratory air, there were important shifts in the polysaccharide solubilities after several weeks. Changes of this type may have entered into our comparisons although all of the samples were held in sealed containers.

TABLE III

DIURNAL VARIATIONS IN THE CARBOHYDRATES OF MAIZE LEAVES, JULY 19, 1932
(PERCENTAGE ON BASIS OF GREEN WEIGHT)

TIME	TOTAL SUGARS	ALPHA-EXTRACT	BETA-EXTRACT	ACID-HYDROLYZABLE	TOTAL POLYSACCHARIDES
	%	%	%	%	%
4 A.M.	0.84	1.01	1.83	2.38	5.22
7 A.M.	0.92	0.66	1.73	2.37	4.76
10 A.M.	1.58	0.67	1.82	2.54	5.03
1 P.M.	2.19	0.91	1.86	2.60	5.37
4 P M.	2.65	1.28	2.01	2.68	5.97
7 P.M.	1.82	1.40	1.97	2.63	6.00
10 P.M.	1.19	1.09	1.84	2.88	5.81
1 A.M.	0.83	0.89	2.00	2.78	5.67
4 A.M.	0.73	0.54	1.95	2.67	5.16

The polysaccharides of the stems showed no diurnal variation. On the green weight basis, however, they showed a steady seasonal increase in the percentage of total polysaccharides. This increase was shown to be caused by the loss of water from the maturing pith, thus one would conclude from the data that there were no significant fluctuations in the polysaccharides of maize stalks during the periods of study.

THE RELATION OF TEMPERATURE TO DIURNAL POLYSACCHARIDE VARIATIONS

SPOEHR (**17**) reported that the polysaccharide-sugar ratio in cacti is dependent upon at least two factors: temperature, and water content. LOOMIS and EVANS (**12**) and LOOMIS (**8**) showed that storage of tubers and corms at temperatures around 33° C. resulted in a reduction of the rest period to one-third of the normal duration, and in a change of polysaccharide reserves to soluble carbohydrates. On the other hand, the rest period was broken and starch was changed to sucrose in tulip bulbs stored at 5° C.

TABLE IV

DIURNAL VARIATIONS IN THE CARBOHYDRATES OF MAIZE LEAVES, JULY 15, 1933
(PERCENTAGE ON BASIS OF GREEN WEIGHT)

TIME	TOTAL SUGARS	ALPHA-EXTRACT	BETA-EXTRACT PLUS ACID-HYDROLYZABLE	TOTAL POLY-SACCHARIDES
	%	%	%	%
4 A.M.	0.80	0.65	4.94	5.59
7 A.M.	1.17	0.65	4.66	5.31
10 A.M.	1.57	0.74	4.95	5.69
1 P.M.	2.11	0.84	5.20	6.04
4 P.M.	2.33	1.32	5.37	6.69
7 P.M.	1.78	1.28	5.11	6.40
10 P.M.	1.39	0.99	4.94	5.93
1 A.M.	1.19	1.03	5.00	6.03
4 A.M.	1.15	0.85	4.61	5.46

CURTIS (3) in his studies on solute translocation in plants found that chilling bean petioles to temperatures between 1° and 4° or 6° C. greatly retarded, or stopped, the removal of carbohydrates from leaf blades but that

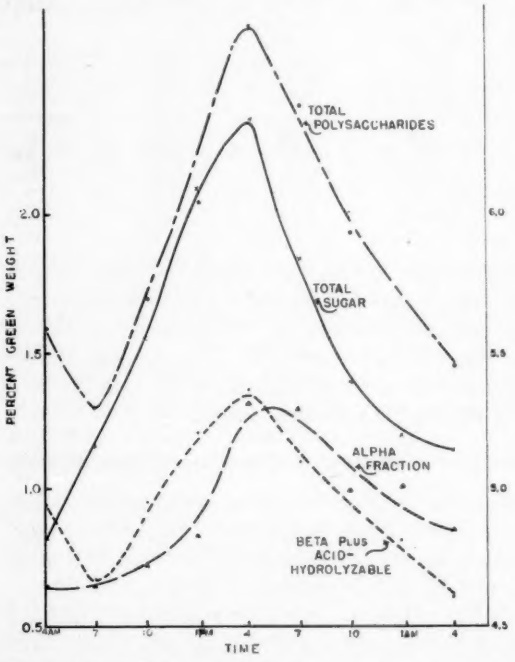


FIG. 11. Diurnal variations in the carbohydrates of maize leaves. July 15–16, 1933.

translocation was not stopped by chilling from 25° to 6° or 8° C. In a later paper CURTIS and HERTY (**4**) found that chilling bean petioles to temperatures between 0.5° and 4.5° C. greatly retarded the removal of carbohydrates from leaf blades but that translocation was not completely stopped by chilling for 17 to 20 hours.

The data of figures 10 and 11 show that in the period from 4 to 7 P.M. while the sugar value was rapidly declining, the total polysaccharide value still showed an upward trend in the leaves. This accumulation of total polysaccharides was accounted for by an increase in the alpha extract. The total sugar content increased in the morning with increased temperature and solar radiation but the maximum sugar content occurred three hours later than the maximum temperature which was reached at 1 P.M. Temperatures seemed to be more closely correlated with diurnal accumulation of sugars than of polysaccharides unless the late afternoon shift to polysaccharides can be interpreted as being the result of falling temperature.

In an investigation of the relationship between temperature and the polysaccharide-sugar balance, experiments were performed upon cut plants held in water and stored at different temperatures. Very little change in carbohydrate content was shown by the different treatments in either total sugars or total polysaccharides.

Samples held at 11° C. were slightly higher in carbohydrates than were those held at 28° C. indicating the utilization of more carbohydrate material in respiration at the higher temperature. There was, however, no evidence of a shift from polysaccharide to sugar or sugar to polysaccharide as a result of the temperature treatment.

SEASONAL VARIATIONS

Our data showed very little change in the percentage of the alpha fraction in either the leaves or stems of maturing maize plants. The acid-hydrolyzable fraction, however, being derived largely from products of differentiation in the aging cells, showed more accumulation. The greatest increase in the leaves was one per cent., calculated on the green weight basis, while the stem polysaccharides increased as much as 4 per cent. from July to August, 1933. It must be kept in mind, however, that the stems become pithy as a result of the loss of water so that their green weight per unit volume decreases rapidly.

When the carbohydrates were calculated as the percentage of residual dry weight, that is, of the dry weight of the tissue after the soluble carbohydrates and the polysaccharides are removed, somewhat different results were obtained. The alpha fraction of both the leaf and stem tissues showed the same general trend while the total polysaccharides of the leaves decreased in the 1932 experiments, and the percentage increase in the 1933 series was lower

than when calculated on the green weight basis. Total stem polysaccharides on August 29, 1932 were about 400 per cent. of their value on July 19 on the green weight basis; when estimated on the residual dry weight basis, however, the August 29 value was only 104 per cent. of that obtained on July 19. The variation in the carbohydrate content of this pithy tissue with its rapidly changing water content should be more accurately expressed on the basis of residual dry weight (total dry weight less carbohydrates) than on the basis of either fresh weight or total dry weight. If this viewpoint is correct we may conclude that hemi-cellulose materials are not deposited in the vegetative cells of maturing corn plants; particularly not in the pith cells of stalks.

Effect of defruiting upon the polysaccharides of maize

Willaman *et al.* (**19**) and Loomis (**11**) have shown that removing the ear caused sugar to accumulate in maize plants. There was likewise a marked seasonal accumulation of sugar in our plants from which the fruits had been removed. In spite of this accumulation of sugar, relatively little accumulation of the alpha extract or total polysaccharides was observed in the stems of defruited plants. Only the alpha fraction in the leaves showed an accumulation which amounted to about double the quantity found in the check leaf tissue.

A comparison of the different polysaccharide fractions in the leaves and stems of fruiting maize plants in the soft dough stage, and of plants from which the ears were removed at silking time was made. The data are somewhat meager as they represent only 4 collections taken rather late in the season and following an abnormally low temperature during the previous night. They should, however, be adequate to show whether the surplus carbohydrates of the defruited plants are stored in the stalk. Rather surprisingly there is no marked evidence of such storage, neither the diurnal variations nor the actual percentage values of the polysaccharides in the defruited plants being strikingly different from those of the check plants. Loomis (**11**) has reported that the stalks of defruited maize plants were frequently heavier than normal although the total weight of stalk and grain in the checks was much greater than that of the defruited plant. He reported, also, sucrose accumulations of 3 or more per cent. of the green weight in the defruited stalk above the values in control stalks. Our analyses, shown in table V, indicate increases of less than one per cent. in alpha polysaccharides, with much smaller increases in the other two fractions. A well developed ear of maize weighs as much as the vegetative plant and the observed accumulations of polysaccharides in the stalk must be contrasted with the normally heavy storage of starch in the grain. The conclusion that the stalks of defruited maize do not replace the ear as storage organs seems to be justified.

TABLE V

POLYSACCHARIDE ANALYSES OF CONTROL AND DEFRUITED MAIZE PLANTS
(PERCENTAGE ON BASIS OF GREEN WEIGHT)

TIME	ALPHA FRACTION		BETA FRACTION		ACID HYDROLYZABLE	
	CHECK	DEFRUITED	CHECK	DEFRUITED	CHECK	DEFRUITED
	%	%	%	%	%	%
LEAVES						
4 A.M.	1.11	2.65	0.54	0.74	5.54	4.44
10 A.M.	1.23	1.83	0.41	0.68	5.47	5.44
4 P.M.	1.53	2.47	0.53	0.83	5.54	5.59
10 P.M.	2.02	2.35	0.63	0.71	5.71	5.92
STEMS						
4 A.M.	0.73	1.11	0.25	0.28	5.20	5.60
10 A.M.	0.51	1.36	0.23	0.35	4.92	5.17
4 P.M.	0.41	1.17	0.19	0.31	5.78	5.82
10 P.M.	0.57	1.48	0.29	0.39	5.36	6.01

Summary

1. The polysaccharides of vegetative maize tissues have been separated into 4 fractions on the basis of solubility. The first fraction is readily soluble in cold 10 per cent. alcohol and may constitute one or two per cent. of the green weight of the tissue. The second fraction is slowly soluble in boiling water; the third fraction is quickly removed by boiling 1 + 20 HCl and the residual fraction, mainly cellulose, is resistant to hot 1 + 20 acid. A fifth fraction may have been present in the neutral lead acetate precipitates from the water extracts.

2. The solubility reactions and hydrolysis curves of the two cleared, water-extractable materials, designated as the alpha and beta fractions or extracts, corresponded to those of dextrins of medium and high molecular weight respectively. Copper reduction methods indicated that the hydrolysates were free of fructose. Osazone reactions of the alpha hydrolysate indicated impure maltose, while those of the beta fraction indicated impure glucose. Although more work is needed to characterize these polysaccharides, they appear to be mixtures of dextrin with other water-soluble gums.

3. The acid-hydrolyzable fraction was commonly larger than the total of the two water-soluble fractions. A reasonably sharp endpoint for the hydrolysis was obtained after 3 hours at 100° C. or 1 hour at 120° C. with 1 + 20 HCl. This fraction showed no significant daily or seasonal variations after the plants reached the silking stage, except as the results of the pith analyses affected by the loss of water from the pith of maturing cornstalks. When these data were calculated to a sugar- and dextrin-free dry weight basis the apparent late season increases disappeared.

4. No starch has been found in any vegetative tissue of maize except for

a few grains near the bundles of stunted seedlings. Starch does not appear to be a constituent of the growing or maturing plant.

5. The alpha-polysaccharide fraction showed diurnal fluctuations in the leaves which were nearly half as great as the sugar fluctuations and lagged approximately 3 hours behind them. Beta-fraction variations were inconsistent and may have been caused by secondary changes within the ground sample. Acid-hydrolyzable and residual materials showed a seasonal accumulation on the green weight basis which disappeared when calculations were based on residual dry weights.

6. Removing the ear at silking resulted in alpha-fraction accumulations of less than one per cent. of the green weight and in smaller accumulations of the other fractions. The maize stalk is not an effective storage organ.

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A STUDY OF DEVELOPMENTAL CHANGES IN COMPOSITION OF THE MACADAMIA[1]

WINSTON W. JONES

(WITH SIXTEEN FIGURES)

Introduction

In recent years the macadamia [*Macadamia ternifolia* F. von Mueller, var. *integrifolia* (Maiden and Betche) Maiden] has assumed increasing commercial importance in the Hawaiian Islands. The Hawaii Agricultural Experiment Station has placed emphasis on establishing varieties and developing improved cultural practices, and toward the latter end it has become desirable to secure knowledge concerning the normal physiological development of this interesting fruit. The present paper sets forth results of a study on the changes in size, weight, and chemical composition of the macadamia fruit from flowering to maturity.

Preliminary work (**7**) has shown that this fruit requires from 215 to 230 days after flowering to reach maturity, and that the mature dry embryo contains about 75 per cent. oil. Very few studies have been reported on the physiological development of perennial oil-seed fruits since THOR and SMITH (**11**) reported on the pecan. Studies on oil-seeds have been largely confined to quick growing crops such as cotton (**4**), flax (**2**), rape, hemp, poppy, and sunflower (**5**). CHEEL and MORRISON (**1**) describe some characteristics of macadamia oil, but, other than a preliminary paper by the writer (**7**), no physiological account of fruit development in macadamia has thus far appeared.

Materials and methods

Fruits were collected at five intervals from flowering to maturity from five well-bearing seedling trees, selected on a basis of apparent uniformity in fruiting characteristics, in a commercial orchard near Honolulu. Figure 1 shows the approximate size of the macadamia at the various stages of maturity selected for analyses.

The first samples were taken approximately 90 days after flowering; before this time the embryos were too small to analyze. One hundred fruits from each tree were used for the first two samplings and fifty for the later samples. The sample from each tree was weighed and the average weight of the fruit determined. Each fruit was sliced through the middle; the diameter of the unhusked nut, the thickness of the shell, and the diameter of the embryo were measured. The material was then separated into husks,

[1] Published with the permission of the Director, Hawaii Agricultural Experiment Station.

shells, and embryos[2] and the fresh weights of the fractions from each tree obtained. Dry weights were obtained after the fractions had been held at 100° C. for 2 to 3 hours and under vacuum at 80° C. for 48 hours.

Preliminary work (7) has shown that the husk and shell change in composition very little during the growth and maturity of the macadamia; thus in the present study chemical analyses were made of the kernel (embryo) only.

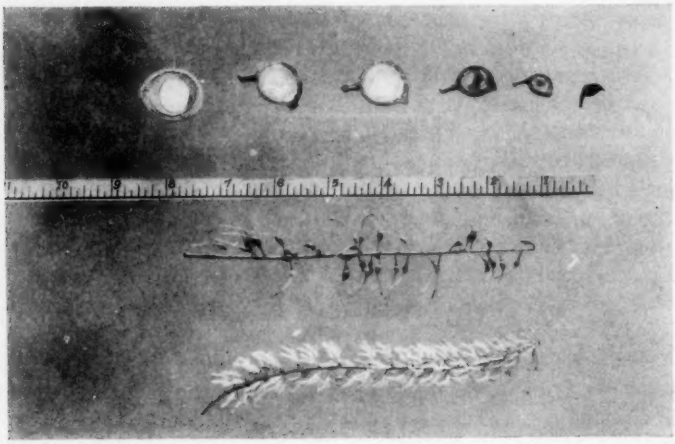

FIG. 1. Lower, macadamia flower cluster; middle, cluster of young nuts; upper, cross section of fruit showing stages at which samples were taken (fruit at extreme right too small for sampling).

OIL

After the first two samples the embryos could not be ground without loss of oil, so the samples were chopped with a sharp knife. The finely chopped material was re-dried in a vacuum oven at 80° C. for 24 hours, and 2.5-gm. aliquots were weighed into alundum cups and extracted for 6 hours with anhydrous alcohol-free ether in a continuous drip extractor. The ether was then removed, and the extract dried, weighed, and reported as oil.

SUGARS

The oil-free residue was extracted with 80 per cent. alcohol. An aliquot of the extract was cleared with neutral lead acetate and potassium oxalate; reducing sugars were determined by the method of STILES, PETERSON, and FRED (10). Sucrose was obtained by the same method after inversion with invertase.

[2] The so-called nut of the macadamia is a true seed; the shell is composed of the outer integument while the edible portion, or kernel, is the embryo (6).

Nitrogen

Soluble nitrogen was determined on aliquots of the alcoholic extract by the reduced-iron method of Pucher, Leavenworth, and Vickery (9), adapted to the micro-Kjeldahl method described by Pregl (8). Insoluble nitrogen was determined on the residue from the alcoholic extraction by the micro-Kjeldahl method of Pregl. At all times the embryo gave a negative test with diphenylamine, indicating the complete absence of nitrate from this organ.

Presentation and discussion of data

The environmental conditions prevailing during the growth of the fruit, including the weekly average day and night temperatures, the weekly average of sunlight intensity, and the weekly rainfall, are shown in figure 2.

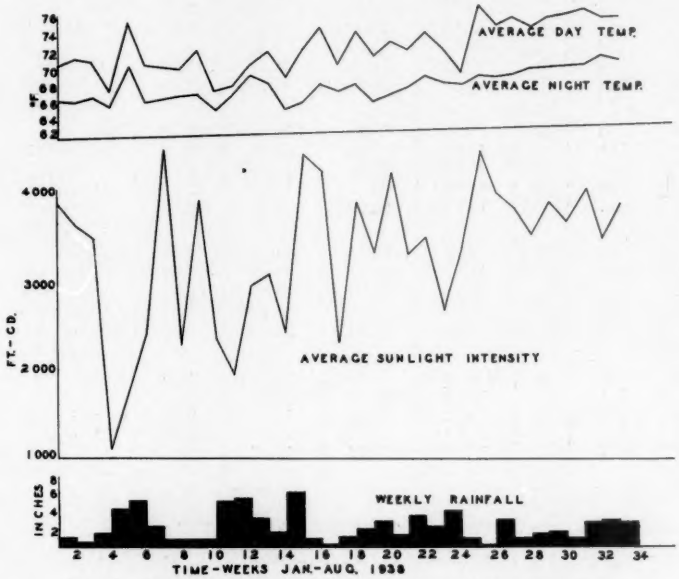

Fig. 2. Rainfall, sunlight, and temperature at Nutridge, Oahu.

The accumulation of dry matter in the macadamia fruit and its parts is shown in tables I and II and figures 3 and 4. The remainder of the analytical data are presented graphically in figures 5 to 16. The graphs show the curves for the individual seedling trees so that the variations are more, in some cases, than if a single clone had been available or if the fruit from several trees had contributed to a single sample. In most instances, however, the trends of development are similar.

TABLE I

SIZE CHANGES IN THE MACADAMIA FRUIT DURING DEVELOPMENT

DATE	DAYS AFTER FLOWERING	TREE NO.	AVERAGE FRESH WEIGHT OF WHOLE FRUIT	AVERAGE DIAMETER OF UNHUSKED NUT	AVERAGE DIAMETER OF EMBRYO	AVERAGE THICKNESS OF SHELL	CONDITION OF SHELL
			gm.	*mm.*	*mm.*	*mm.*	
4–13–38	90	2	3.02	17.92	9.08	2.32	Soft
5– 4–38	111		8.64	24.56	16.68	2.10	"
	111		9.30	25.44	16.88	2.06	Hard—white
5–19–38	126		10.24	26.09	16.86	2.29	" —light brown
7–13–38	185		11.32	26.98	17.38	1.83	" —brown
8–22–38	215		12.37	27.76	17.54	2.15	" —dark brown —nuts mature
4–13–38	90	4	3.61	17.44	9.64	2.12	Soft
5– 4–38	111		7.76	23.68	15.92	2.24	"
	111		8.58	24.44	16.00	2.14	Hard—white
5–19–38	126		10.70	26.91	17.95	2.51	" —light brown
7–13–38	185		12.42	28.24	17.76	2.36	" —brown
8–22–38	215		13.52	28.91	18.20	2.74	" —dark brown —nuts mature
4–14–38	90	6	7.33	24.02	15.92	1.97	Soft
5– 5–38	111		12.92	27.48	18.42	2.13	Hard—white
5–20–38	126		10.29	26.26	17.60	2.08	" —light brown
7–14–38	185		13.72	28.68	18.08	2.12	" —brown
8–23–38	215		13.51	28.70	18.18	2.17	" —dark brown —nuts mature
4–14–38	90	7	6.60	22.14	14.24	2.09	Soft
5– 5–38	111		13.08	28.38	18.42	2.61	Hard—white
5–20–38	126		14.14	28.94	19.00	2.43	" —light brown
7–14–38	185		17.10	30.70	19.62	2.28	" —brown
8–23–38	215		17.26	30.82	20.00	2.32	" —dark brown —nuts mature
4–14–38	90	9	4.32	19.28	11.00	1.75	Soft
5– 5–38	111		13.32	28.70	18.94	2.15	Hard—white
5–20–38	126		13.92	28.78	18.88	2.22	" —light brown
7–14–38	185		14.70	29.64	18.90	1.86	" —brown
8–23–38	215		16.00	30.22	19.76	1.93	" —dark brown —nuts mature

GROWTH OF THE MACADAMIA FRUIT

The data of table I show the changes with time in size and fresh weight of the fruit. In no case had the shells hardened in the 90-day sample; at 111 days it will be noted that all the fruit from trees 2 and 4, although of the same age, were not at the same stage of development. The shell was soft on a part of the fruit and on the remainder the shells had hardened; all of the fruit from trees 6, 7, and 9 had hard shells at 111 days. From table I it is apparent that by the time the shell hardened the fruit had reached approximately 70 per cent. or more of its final fresh weight. After the shell hardened the diameter of the embryo and the thickness of the shell increased only slightly.

TABLE II

DRY WEIGHT CHANGES IN THE MACADAMIA FRUIT

DATE	DAYS AFTER FULL FLOWER	TREE NO.	AVERAGE DRY WEIGHT PER FRUIT OF				RELATION TO TOTAL DRY WEIGHT OF		
			WHOLE FRUIT	HUSK	SHELL	EMBRYO	HUSK	SHELL	EMBRYO
			gm.	*gm.*	*gm.*	*gm.*	%	%	%
4–13–38	90	2	0.59	0.46	0.12	0.01	77.6	20.2	2.2
5– 4–38	111		1.32	0.95	0.48	0.09	62.3	31.7	6.0
	111		2.47	1.06	1.40	0.21	42.9	48.6	8.5
5–19–38	126		3.59	1.17	1.99	0.43	32.6	55.4	12.0
7–13–38	185		5.32	1.58	2.40	1.34	29.7	45.1	25.2
8–22–38	215		6.31	2.00	2.84	1.47	31.7	45.0	23.3
4–13–38	90	4	0.64	0.46	0.15	0.02	72.6	23.8	3.6
5– 4–38	111		1.37	0.80	0.48	0.09	58.4	35.1	6.5
	111		1.49	0.50	0.83	0.16	33.7	55.4	10.8
5–19–38	126		3.32	1.02	1.86	0.44	30.8	55.9	13.3
7–13–38	185		6.37	1.48	3.40	1.49	23.2	53.4	23.4
8–22–38	215		9.38	3.82	3.85	1.71	40.7	41.1	18.2
4–14–38	90	6	1.41	0.92	0.40	0.09	65.4	28.2	6.4
5– 5–38	111		4.28	1.26	2.16	0.86	29.5	50.5	20.0
5–19–38	126		3.87	1.06	1.93	0.88	27.4	49.8	22.8
7–13–38	185		4.48	1.76	0.80	1.92	39.3*	17.9*	42.8*
8–22–38	215		6.59	1.91	2.84	1.84	29.0	43.1	27.9
4–14–38	90	7	0.84	0.52	0.26	0.06	62.0	31.0	7.0
5– 5–38	111		4.96	1.47	2.59	0.90	29.6	52.3	18.1
5–19–38	126		6.08	1.48	3.31	1.29	24.3	54.4	21.3
7–13–38	185		8.68	2.38	4.06	2.24	27.4	46.8	25.8
8–22–38	215		8.89	2.47	4.11	2.31	27.8	46.2	26.0
4–14–38	90	9	0.90	0.69	0.17	0.04	76.8	19.1	4.1
5– 5–38	111		4.08	1.60	1.94	0.54	39.2	47.5	13.3
5–19–38	126		5.40	1.83	2.61	0.96	33.9	48.3	17.8
7–13–38	185		6.21	2.02	2.64	1.55	32.5	42.5	25.0
8–22–38	215		6.90	2.31	2.98	1.61	33.5	43.2	23.3

* It may be seen from table I that the fruits in this particular sample were small with thin shells, hence the seeming discrepancies in these figures.

The data of table II show the changes in dry weight with time in the macadamia fruit, while the graphs of figures 3 and 4 show the moisture changes. It will be noted from table II that there was an increase in total dry weight of the fruit and its parts from the first to the last sampling date (except for the 4th sample in tree no. 6 in which the sampled nuts were small (see table I). This is in contrast to the total fresh weight shown in table I, in which the increase was slight after the shells became hard and light brown. Figures 3 and 4 indicate a marked decrease in moisture content of the embryo and shell, which accounts for the lack of increase in total fresh weight even though the dry weight is increasing.

CHEMICAL CHANGES IN THE MACADAMIA EMBRYO

OIL.—Figures 5 and 6 present the data on oil formation in the macadamia embryo. During the first 90 days after flowering there was very little

oil formation and only slight enlargement of the embryo. Thereafter the embryo began to enlarge, and oil formation was rapid so that 40 days later, or about 130 days after flowering, the oil had reached more than half of the final concentration. During the last 30 days before maturity there was very little increase in the percentage concentration of oil. There is much more variation between the individual trees on the basis of total oil per fruit than

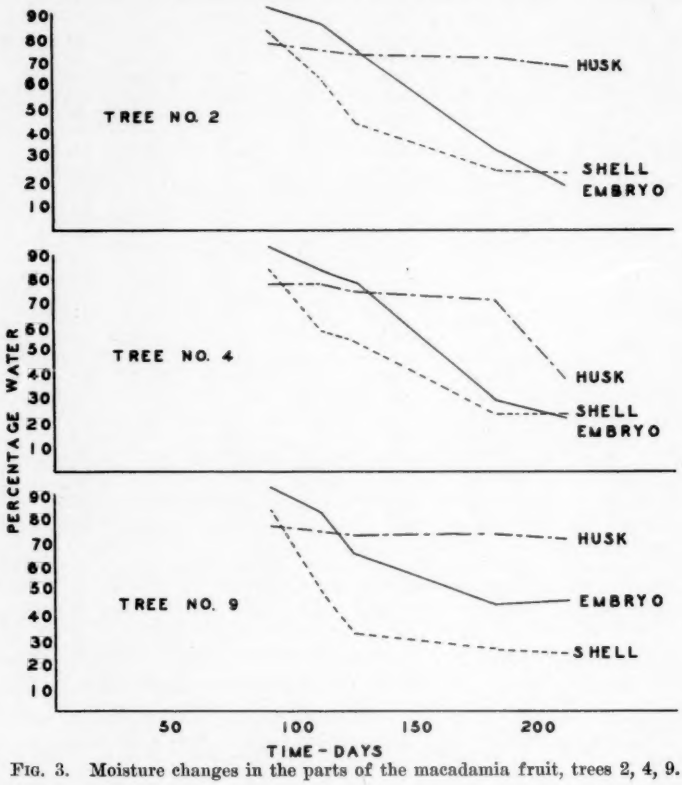

FIG. 3. Moisture changes in the parts of the macadamia fruit, trees 2, 4, 9.

there is on the percentage basis, as seen from a comparison of figures 3, 4 and 5. This difference is attributable to the variations in the size of the fruit.

In three cases the quantity of oil per embryo increased until the time of harvest, while in two cases there were slight drops. In considering an average of the five trees, however, the total oil per embryo showed an almost straight line increase from the beginning of oil formation at 90 days after flowering until maturity 215 days after flowering. This uniform gain is in

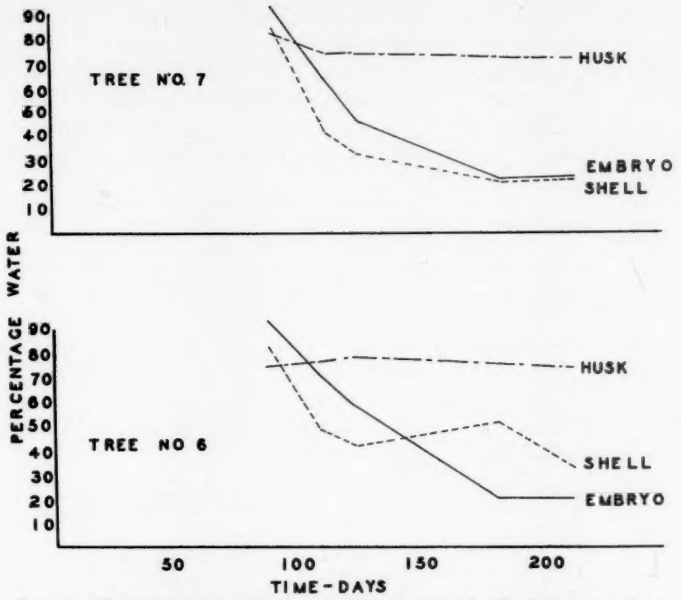

FIG. 4. Moisture changes in the parts of the macadamia fruit, trees 6, 7.

contrast to the pecan (11) which shows no increase in amount of oil per nut during the 25 days preceding harvest. Why the total oil per embryo of two trees should have decreased is not clear, although it is possible that the phenomenon will prove to be related to the beginning of germination of the embryo. Since a part of the fruit for the last sample was harvested from the ground, in the commercial manner, and since the macadamia germinates

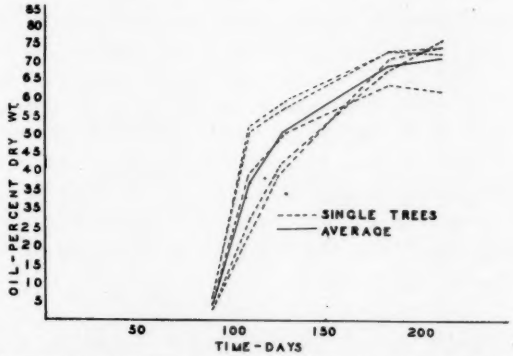

FIG. 5. Changes in oil content during development of the macadamia embryo.

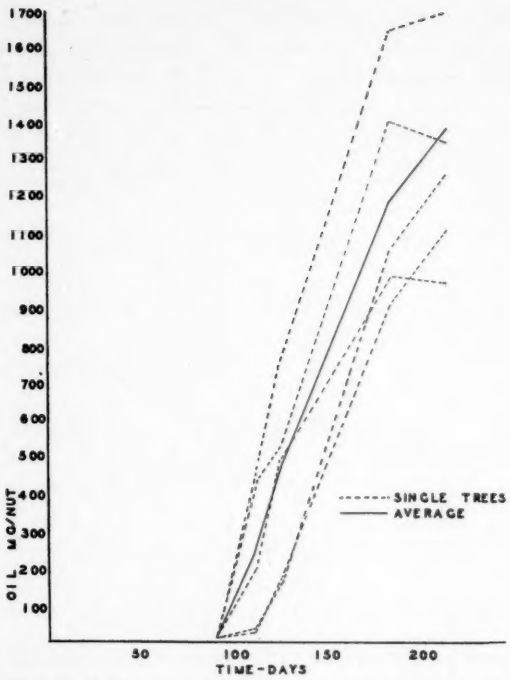

FIG. 6. Changes in oil content during development of macadamia embryo.

readily (in some cases before the nuts drop), some germination may have begun in these two samples although no detectable growth had occurred.

OIL-FREE SOLUBLE SOLIDS

Figures 7 and 8 show the changes in oil-free soluble solids of the embryo. On the dry weight percentage basis, there was a rapid decrease from the 90th to the 111th day and then a more gradual decrease until the 215th day, at which time the fruits were considered mature. On the basis of milligrams per embryo, however, the oil-free soluble solids showed an increase until the fourth sampling date, 185 days after flowering, followed by a slight decrease between the 185th and 215th days. The variation between these two figures demonstrates the danger of expressing data on a percentage of dry weight basis, especially when there is a second factor (the rapidly increasing oil in the present case).

SUGARS

Figures 9, 10, 11, and 12 show the sugar changes in the macadamia embryo during development. From figure 9 it is noted that the percentage

of reducing sugar fell to a low level at 126 days and remained low until the 185th day; thereafter a slight increase occurred to the last harvest at 215 days. The explanation for this increase in reducing sugar is not clear. As discussed under oil, it might have been caused by the beginning of germination. Another plausible explanation is that, after the rate of formation

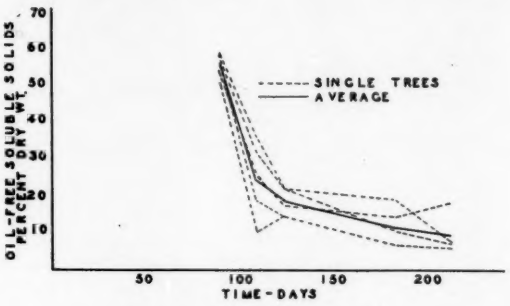

FIG. 7. Oil-free soluble solids.

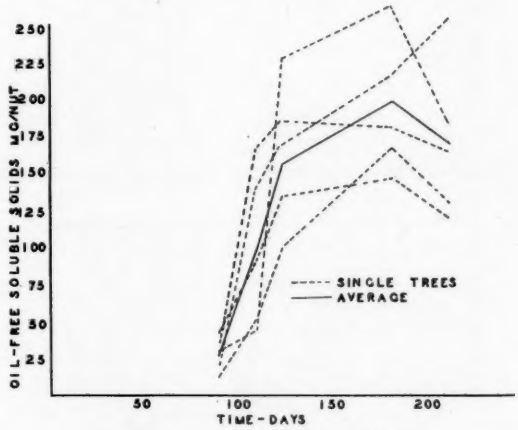

FIG. 8. Oil-free soluble solids.

of oil has decreased, the movement of carbohydrates into the fruit is not retarded proportionally so that reducing sugars accumulate in the embryo. No starch was found in the embryo at any time. Figure 11 shows the quantity of reducing sugar per embryo. The average for the five individual trees shows an increase in total amount up until the 126th day. Beginning at 126 days there was a decrease to the 185th day and then a marked increase to maturity.

Figure 10 shows that sucrose increased, on a percentage basis, until about the 126th day and then gradually decreased to a minimum at maturity. The quantity of sucrose per embryo (fig. 12) also increased until about the 126th

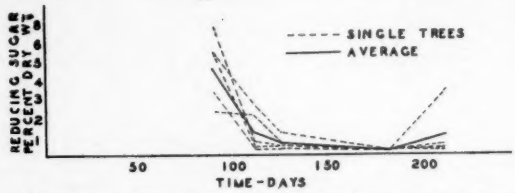

FIG. 9. Changes in reducing sugar content during development of the macadamia nut.

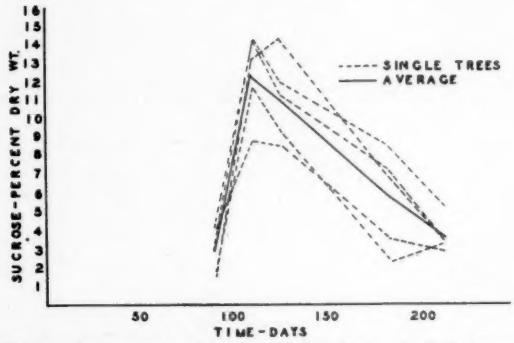

FIG. 10. Changes in sucrose content during development of the macadamia nut.

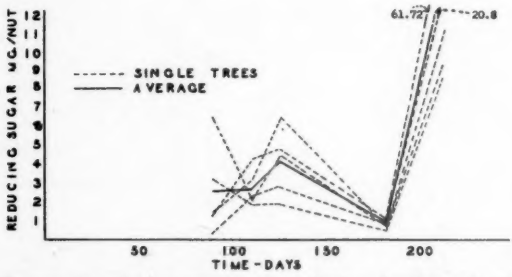

FIG. 11. Changes in reducing sugar content during development of the macadamia nut.

day after flowering, but the total amount remained more or less constant until the 185th day, after which there was a slight decrease to maturity.

NITROGEN

Figures 13 to 16 present the data on changes in nitrogen during development of the embryo. Nitrate-free soluble nitrogen maintained a constant

low percentage value after 126 days but on the basis of quantity per embryo it reached a minimum at 111 days and then increased to the 185th day. There was a slight drop between the 185th and 215th days. Insoluble nitrogen (fig. 14), on a percentage basis, reached a maximum at 126 days, de-

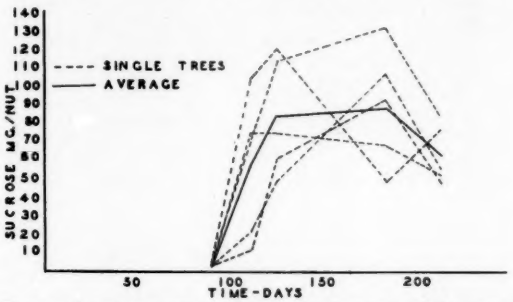

FIG. 12. Changes in sucrose content during development of the macadamia nut.

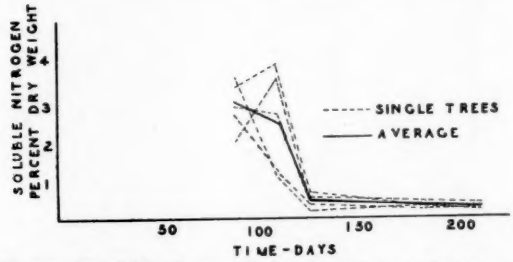

FIG. 13. Changes in soluble nitrogen during development of the macadamia nut.

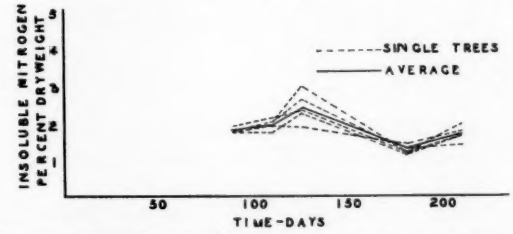

FIG. 14. Changes in insoluble nitrogen during development of the macadamia nut.

creased to the 185th day, then increased slightly to maturity. When the total weight of insoluble nitrogen per embryo (fig. 16) is considered, there was an increase from the first to the last sample. The total amount of nitrogen (insoluble plus soluble) per embryo also increased throughout the development of the fruit.

Summary

1. A study was made of the physiological development of the macadamia embryo. No chemical analyses are presented for any parts of the fruit other than the embryo, since preliminary work has shown very little change in the husk and shell except during early stages.

2. The development of the fruit is characterized by two distinct periods:

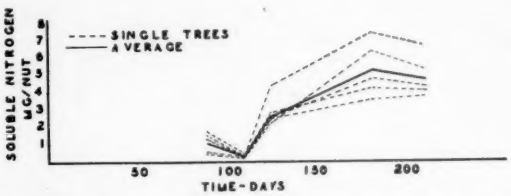

FIG. 15. Changes in soluble nitrogen during development of the macadamia nut.

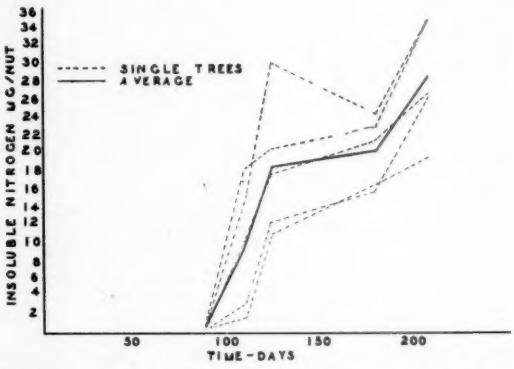

FIG. 16. Changes in insoluble nitrogen during development of the macadamia nut.

the first is from flowering to the end of 90 days, during which very little oil is formed and the embryo does not enlarge sufficiently for analysis; the second is from 90 days to maturity, a period of about 125 days, during which oil is formed and the major expansion of the embryo occurs.

3. The total amount of sugar present increases during the early oil formation but decreases as maturity is attained.

4. Protein synthesis occurs during the same period as oil synthesis.

Thanks are due MR. BRUCE COOIL for assistance in collecting the samples and to MR. HISASHI KUBOTA for a part of the analytical work.

Appreciation is herewith expressed to the Hawaiian Macadamia Nut

Company for their kind cooperation in making their macadamia grove at Nutridge, Oahu, available to the Station for experimental purposes.

HAWAII AGRICULTURAL EXPERIMENT STATION
HONOLULU, T. H.

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THE EFFECT OF HOST NUTRITION ON CONCENTRATION OF TOBACCO-MOSAIC VIRUS

ERNEST L. SPENCER

(WITH ONE FIGURE)

Introduction

It is well known that variations in the nitrogen nutrition of tobacco plants drastically alter their susceptibility to infection with tobacco-mosaic virus (**16**) and the rate of spread of virus in the tissues (**17**). Little attention has been given to the possible effects of nitrogen variations on the virus entity itself. In order to determine whether variations in the nitrogen content of the nutrient solution would bring about changes in the virus concentration[1] within the plant, tests were made of the activity of expressed juice from mosaic-diseased plants receiving different amounts of nitrogen. The results of these tests are reported in this paper.

Materials and methods

Turkish tobacco (*Nicotiana tabacum* L.) was used as the experimental host plant. Uniform seedlings in the 3-leaf stage were selected and transplanted to washed quartz sand or composted soil in 4-inch porous clay pots. The temperature of the greenhouse in which the plants were grown was held between 70° and 80° F. during the day and between 70° and 75° F. at night. The sand-grown plants were supplied with nutrient solutions containing either 1, 20, or 200 mg. of nitrogen per 100 ml. of solution, according to methods previously described (**17**). The medium-nitrogen level produced vigorous plants comparable to those grown in composted soil. The low and high levels were used in order to test the effect of a restricted supply of nitrogen and the effect of an amount in excess of that required for good growth. The high level retarded normal plant development but produced no other evidence of toxicity.

Nutrient application was started on the third day after transplanting. Between applications the plants were watered whenever necessary. The composition of the 3 nutrient solutions is shown in table I. In addition to the salts listed, boron and manganese were added to all solutions at a concentration of 0.5 p.p.m. of each as H_3BO_3 and $MnSO_4 \cdot 2H_2O$, respectively. Representative plants in each group were inoculated with ordinary tobacco-mosaic virus, on the third day after transplanting, by rubbing the leaves

[1] The expression virus concentration or content as used in this paper refers only to the virus content in the expressed juice. While changes in the virus content in the juice probably parallel similar changes in the entire plant, this relationship has yet to be shown.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

STOCK SOLUTIONS (0.5 MOLAR)	VOLUME OF STOCK SOLUTIONS PER LITER OF NUTRIENT SOLUTION		
	LOW-NITROGEN SOLUTION	MEDIUM-NITROGEN SOLUTION	HIGH-NITROGEN SOLUTION
	ml.	*ml.*	*ml.*
KH_2PO_4	12.9	12.9	.....
$Ca(NO_3)_2$	0.5	11.0	63.0
$MgSO_4$	4.0	4.0	4.0
$(NH_4)_2SO_4$	0.2	3.3	.....
$CaCl_2$	11.0	.....	.....
$NH_4H_2PO_4$	.....	.....	12.9
NH_4NO_3	.....	.....	67.0
KNO_3	.....	.....	12.9

with a cheese cloth pad moistened with infectious juice. Typical symptoms of mosaic appeared about 5 days later on all plants inoculated. The experiment was carried out during October and November. The results reported are comparable with those obtained in earlier preliminary experiments.

Experimentation

GROWTH OF PLANTS

Ten representative healthy and diseased plants from each treatment were harvested at 15, 25, and 35 days following inoculation. The heights of the plants and green weights of leaves were recorded at time of harvest. Dry weights were determined after the leaves had been heated for 10 hours at 100° C. and then dried to constant weight at 80° C. The relative growth of the plants at the first, second, and third harvests is illustrated in figure 1. The 4 plants on the left in each row were healthy, the 4 on the right were diseased. In each group of 4 plants, the first, from left to right, had received the low-nitrogen solution, the second the medium-nitrogen solution, and the third the high-nitrogen solution. The fourth plant in each group was the control grown in composted soil.

Table II gives the growth data obtained on the diseased plants at each of the 3 harvests. When harvested the diseased plants were smaller and weighed from 40 to 60 per cent. less than healthy plants on similar nutrient treatments. After 25 days, the diseased plants in sand on the medium-nitrogen treatment were practically the same size as the control plants in soil. After 35 days, however, the plants in sand were much taller and weighed about 30 per cent. more than the soil-grown plants. At all harvests the plants on the high-nitrogen treatment were smaller than those grown in soil. The low-nitrogen plants remained practically dormant over the entire

period of 35 days. It is of interest to note the percentage dry weight of the leaves on the several treatments, as recorded in table II. The high-nitrogen plants had the highest percentage dry weight and the control plants in soil had the lowest. This difference in moisture content is probably due to the

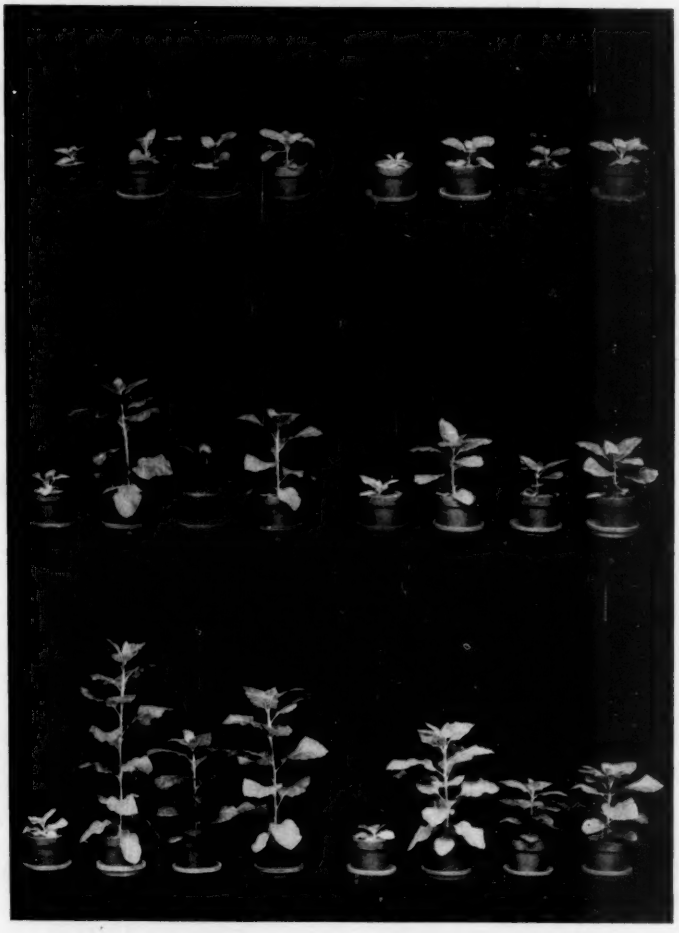

FIG. 1. Typical tobacco plants at time of harvest after 15, 25, and 35 days of treatment, respectively, from top to bottom. The 4 plants on the left in each row were healthy, the other 4 diseased with tobacco mosaic. In each group, the 4 treatments were low nitrogen, medium nitrogen, high nitrogen, and control in soil, respectively, from left to right. (Photograph by J. A. CARLILE.)

TABLE II

EFFECT OF NITROGEN ON GROWTH OF DISEASED TOBACCO PLANTS HARVESTED AT 3 INTERVALS FOLLOWING INOCULATION

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULATION	GROWTH RECORDS AT TIME OF HARVEST			PERCENTAGE DRY WEIGHT OF LEAVES
		HEIGHT	GREEN WEIGHT OF LEAVES	DRY WEIGHT OF LEAVES	
	days	*cm.*	*gm.*	*gm.*	%
Low	15	1*	1.8*	0.11*	6.1
Medium	15	2	6.0	0.39	6.5
High	15	2	4.9	0.40	8.2
Control	15	4	7.0	0.43	6.1
Low	25	1	3.2	0.24	7.5
Medium	25	12	19.8	1.48	7.5
High	25	4	8.0	0.72	9.0
Control	25	12	19.5	1.17	6.0
Low	35	2	4.1	0.30	7.3
Medium	35	31	36.4	2.67	7.3
High	35	12	18.3	1.64	9.0
Control	35	19	28.3	1.85	6.5

* Average of 10 plants in each group.

high osmotic pressure exerted by the salts in the high-nitrogen solution, making it difficult for the plant to take up water from the solution. The osmotic pressure of the soil solution was probably quite low.

VIRUS ACTIVITY OF JUICE

Diseased plants similar to those used for growth measurements were cut at each harvest and the leaves frozen in a cold room held at $-14°$ C. On the following day they were thawed and minced in a food chopper. The juice was expressed from the pulp through cheese cloth, under pressure from a small hand press. The extracted juice was placed in sealed tubes and kept frozen at $-14°$ C. until used. Before being tested for virus activity, an aliquot of the juice was thawed and cleared of all insoluble materials by low-speed centrifugation.

The virus activity of the cleared juice was determined by the local-lesion method (**8**) modified, as suggested by SAMUEL and BALD (**14**), so that 2 samples were tested on opposite halves of the same leaf, thereby eliminating much of the variation attributable to differences in the susceptibility of individual plants. *Phaseolus vulgaris* L. var. Early Golden Cluster was used as a test plant, since PRICE (**12**) has shown that plants of this variety are suitable for measuring concentration of tobacco-mosaic virus. Nine- or 10-day-old bean plants were selected for uniformity in the size and development of primary leaves. These leaves were inoculated by rubbing the upper surface of each with a sterile pad of cheese cloth moistened with the solution

to be tested. Only 4 samples were compared at any one time, making it possible to test each sample once on every plant. Dilutions of the cleared juice were made with 0.1 M potassium phosphate buffer at pH 7.0. The lesions were counted 5 days after inoculation.

The total numbers of lesions produced on 36 half-leaves by each virus sample at dilutions of 1/100, 1/300, and 1/1000 are shown in table III.

TABLE III

VIRUS CONCENTRATION OF JUICE EXTRACTED FROM DISEASED TOBACCO PLANTS RECEIVING DIFFERENT AMOUNTS OF NITROGEN

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULATION	VOLUME OF JUICE EXTRACTED PER PLANT	NUMBER OF LESIONS ON 36 HALF LEAVES AT A DILUTION OF			RELATIVE VIRUS CONTENT* PER	
			1/100	1/300	1/1000	ML. OF JUICE	PLANT
	days	*ml.*					
Low	15	1.4	698	555	438	0.4	0.12
Medium	15	4.8	1252	764	536	1.4	1.5
High	15	2.8	1186	807	560	1.5	0.9
Control	15	4.5	1064	702	507	1.0	1.0
Low	25	1.8	350	306	177	0.04	0.005
Medium	25	16.8	946	830	566	2.0	2.3
High	25	4.8	1044	914	674	3.1	1.0
Control	25	14.3	894	720	405	1.0	1.0
Low	35	2.6	66	113	59	0.03	0.004
Medium	35	27.5	900	598	186	1.4	2.0
High	35	11.7	945	818	291	2.5	1.5
Control	35	19.4	717	496	177	1.0	1.0

* Calculated from dilution curves as described in text.

There are several ways in which these lesion count data may be used to estimate the difference in concentration between virus samples tested at any one time. A comparison of the number of lesions produced at any one dilution shows which sample is most concentrated. The magnitude of the differences in concentration cannot be expressed by the differences in number of lesions, because, as is well known, the numbers of lesions produced are not directly proportional to the dilution of virus used for inoculation. When lesion count data at several dilutions are plotted on logarithmic paper, however, the points will lie along a straight line with a slope less than unity. The actual value of the slope will vary, depending upon the conditions under which the data are obtained. Consequently, it is necessary to take this fact into consideration in obtaining a mathematical comparison of the relative virus content of the samples. The method of calculation finally adopted may be illustrated with the data recorded at the first harvest 15 days after inoculation.

A straight line of the form

$$\text{Log } Y = b + a \log X$$

where Y is the number of lesions, X is the dilution, and a and b are constants, was fitted to the data from all 4 virus samples at each of the 3 dilutions. The equation obtained was:

$$\text{Log } Y = 3.612 + 0.304 \log X$$

The slope of this line, 0.304, may be regarded as the mean for all 4 virus samples. In a similar manner, straight lines were fitted to the data for each of the 4 samples. The values obtained were:

Low-nitrogen treatment	$\text{Log } Y = 3.247 + 0.202 \log X$
Medium-nitrogen treatment	$\text{Log } Y = 3.818 + 0.367 \log X$
High-nitrogen treatment	$\text{Log } Y = 3.722 + 0.326 \log X$
Control	$\text{Log } Y = 3.659 + 0.321 \log X$

The slopes of these 4 lines are similar, varying between 0.202 and 0.367. It is probable that this variation is mostly attributable to experimental error, inasmuch as the same virus was used throughout and inoculations were made at the same time. Using the mean slope, 0.304, and the midpoints of each of the 4 lines (*i.e.*, 2.742, 2.900, 2.907, and 2.856, respectively), the following equations were calculated:

Low-nitrogen treatment	$\text{Log } Y = 3.502 + 0.304 \log X$
Medium-nitrogen treatment	$\text{Log } Y = 3.660 + 0.304 \log X$
High-nitrogen treatment	$\text{Log } Y = 3.667 + 0.304 \log X$
Control	$\text{Log } Y = 3.616 + 0.304 \log X$

From these equations it is possible to calculate for any value of Y the corresponding value of X. For example, the degree of dilution (X) necessary to produce a given number of lesions (Y), such as 700, would be for the low-nitrogen treatment, 1/145; medium-nitrogen treatment, 1/480; high-nitrogen treatment, 1/506; and control, 1/344. The virus content of each virus sample when expressed according to the relative dilutions needed to produce a given number of lesions, was thus found to be 0.4, 1.4, 1.5, and 1.0, respectively.

In a consideration of table III, it is possible to compare with one another only those treatments that occur within any one of the 3 groups, because in the virus activity studies the concentration of only 4 treatments was tested at any one time. At 15 days after inoculation, the virus concentration (content per ml. of juice) of high-nitrogen plants did not differ significantly from that of plants on the medium-nitrogen treatment. With older plants, however, the virus concentration produced on the high-nitrogen treatment was appreciably higher than that produced on the medium-nitrogen treatment. Moreover, the plants in sand receiving nitrogen yielded juice with a much higher virus concentration than that from control plants grown in

soil. Another point of interest is the low virus concentration in mature plants deficient in nitrogen. The amount of virus in this juice was only about 1/30 that in juice from plants grown in soil and about 1/80 that of plants receiving the high-nitrogen solution. In general, the data show that the amount of virus extractable per ml. of juice is definitely correlated with the amount of nitrogen supplied to the plant. That is to say, plants deficient in nitrogen had a low concentration of virus, while plants receiving an ample supply had a high concentration.

The effect which these nitrogen levels have on the total amount of virus in the expressed juice from each plant is shown in the last column of table III. The virus content per plant was calculated by multiplying the relative virus concentration by the total number of ml. of juice extracted from each plant. These data show that the nitrogen treatments in sand produced plants with a virus content higher than that produced in soil-grown plants. The virus content was not dependent primarily upon the actual growth of the plants. That is to say, the plants in soil were nearly twice the size of those in sand on the high-nitrogen treatment, yet they had only about $\frac{2}{3}$ as much total virus in their expressed juice.

The data of table III do not allow a direct comparison between virus concentration of plants on the same treatment but of different ages, since virus activity of these groups was determined at different times. For the purpose of making such a comparison, additional tests were carried out. The experimental data are presented in table IV. In each group, the virus

TABLE IV

CONCENTRATION OF VIRUS IN JUICE EXTRACTED FROM DISEASED TOBACCO PLANTS HARVESTED 15, 25, AND 35 DAYS FOLLOWING INOCULATION

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULATION	NUMBER OF LESIONS ON 36 HALF LEAVES AT A DILUTION OF			RELATIVE VIRUS CONTENT PER	
		1/100	1/300	1/1000	ML. OF JUICE	PLANT
	days					
Low	15	877	472	325	1.0	1.0
Low	25	535	187	67	0.2	0.3
Low	35	343	124	85	0.15	0.3
Medium	15	1745	1145	543	1.0	1.0
Medium	25	1343	893	513	0.6	2.1
Medium	35	1193	869	570	0.6	3.4
High	15	1275	778	574	1.0	1.0
High	25	1339	968	770	2.0	3.4
High	35	1187	1014	654	1.5	6.3
Control	15	1683	769	654	1.0	1.0
Control	25	1607	1111	658	1.3	4.1
Control	35	1285	877	608	0.8	3.4

concentration of the juice at the first harvest is taken as unity. Only 3 samples were tested at any one time. On the low- and medium-nitrogen treatments, the virus concentration was higher 15 days after inoculation than it was after 25 or 35 days. In the high-nitrogen plants, the virus concentration after 25 days was double that of the first harvest 10 days earlier. Ten days later, however, the virus concentration had decreased about 25 per cent. In the control plants in soil, the virus concentration reached a maximum in about 25 days and then decreased about 50 per cent. during the next 10 days.

The effect which these nitrogen treatments have on the total amount of virus in extracted juice from leaves of each plant is shown at the right in table IV. In low-nitrogen plants, the virus content of the expressed juice decreased about $\frac{2}{3}$ during the 20 days following the first harvest, even though the plants more than doubled in size during this period (table II). In the control plants and those on the medium-nitrogen treatment, the virus content increased about 3 times, whereas growth increased 4 or 5 times in the 20-day interval between the first and third harvests. In the high-nitrogen plants, the virus increased over 6-fold, whereas growth increase was only 4-fold during this 20-day period.

Nitrogen content of juice

In order to determine whether there was any relationship between the virus concentration and nitrogen content of the extracted juice, aliquots of the juice were analyzed for total nitrogen and protein nitrogen. The aliquots used were from the same lots of juice used in the virus activity studies described above. The juice was thawed and then cleared of all insoluble materials in the same way by low-speed centrifugation. Analyses were also carried out on aliquots from juices of healthy plants.

Total nitrogen was determined by a modification of the method outlined by Ranker (**13**). The method, adapted to micro-determinations, was as follows: Two ml. of plant juice were placed in a 125-ml. Kjeldahl flask and evaporated to dryness in an oven held at 80° C. Salicylic acid (0.15 gm.) and concentrated H_2SO_4 (4 ml.) were added and the mixture allowed to stand overnight with occasional shaking. Zinc dust (0.15 gm.) and a few alundum chips were then added and the mixture boiled gently over a microburner for about 10 minutes. Kjeldahl nitrogen was then determined by the method of Folin and Farmer (**7**) as modified by Northrop (**10**) and Lauro (**9**). To the reduced solution was added 1 gm. of K_2SO_4 and 2 drops of SeOCl and the mixture digested for at least 5 minutes after the contents had become clear or straw-colored. Thirty ml. of water were added and the solution allowed to cool. After the addition of 12 ml. of a 50 per cent. NaOH solution, nitrogen as ammonia was distilled over into 0.02 N standard

acid. With methyl red as an indicator, all titrations were made with 0.02 N NaOH to the complete disappearance of any red tinge.

Protein nitrogen was determined as follows: 4 ml. of juice were treated with 4 ml. of hot 5 per cent. trichloroacetic acid. The suspension was immediately cooled and then centrifuged. The denatured protein was dissolved in 1 ml. of 0.2 N NaOH and reprecipitated with 1 ml. of cold 10 per cent. trichloroacetic acid. Following centrifugation, the pellet was dissolved in 1 ml. of 0.2 N NaOH and transferred to a 125-ml. Kjeldahl flask. Kjeldahl nitrogen was then determined, as already described, by adding concentrated H_2SO_4, alundum chips, K_2SO_4, etc.

From the data in table V, it is obvious that there is a decided variation, not only in the total-nitrogen but also in the protein-nitrogen content of the

TABLE V

NITROGEN ANALYSES OF JUICE FROM FROZEN HEALTHY AND DISEASED TOBACCO PLANTS HARVESTED 15, 25, AND 35 DAYS AFTER INOCULATION

Nitrogen level	Time of harvest: days after inoculation	Average green weight of leaves		Nitrogen per ml. of juice			
				Total nitrogen		Protein nitrogen	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	days	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*
Low	15	3.3	1.8	0.20	0.35	0.01	0.21
Medium	15	12.8	6.6	1.26	1.46	0.22	0.60
High	15	7.4	3.9	2.34	2.33	0.14	0.46
Control	15	12.1	6.0	1.04	1.13	0.15	0.42
Low	25	4.9	2.3	0.18	0.28	0.003	0.10
Medium	25	28.6	21.6	0.61	1.15	0.15	0.52
High	25	17.8	6.7	2.64	3.36	0.13	0.73
Control	25	37.2	17.7	0.68	0.89	0.09	0.40
Low	35	6.7	3.7	0.20	0.25	0.005	0.04
Medium	35	49.2	37.7	0.63	1.06	0.15	0.57
High	35	35.7	17.7	3.08	3.80	0.14	0.77
Control	35	62.5	26.0	0.46	0.65	0.10	0.39

juice from diseased plants, depending on the nutrient treatment. The total-nitrogen content (per ml. of juice) was correlated with the amount of nitrogen supplied to the plants. The nitrogen content of all plants except those on the high-nitrogen treatment was higher at the first harvest 15 days after inoculation than at either of the 2 subsequent test periods. With the high-nitrogen plants, however, the nitrogen content was higher 35 days after inoculation than it had been after 25 or 15 days. The plants on the low-nitrogen treatment had very little nitrogen in their extracted juice.

The protein-nitrogen fraction showed the same general correlation as exhibited by the total-nitrogen content. The only exception was the low

protein-nitrogen content of the high-nitrogen plants at the first harvest. It is of interest to note that, with the exception of the plants on the high-nitrogen treatment, the protein nitrogen accounted for about 50 per cent. of the total nitrogen present in the juice. In the high-nitrogen plants, however, the protein nitrogen accounted for only 20 per cent. of the total nitrogen, indicating that there was present in these plants a much larger reserve of inorganic nitrogen available for assimilation.

Although the nitrogen content in juice from healthy plants was lower than that in juice from diseased plants on corresponding treatments, the variations among treatments were of the same general order. In healthy juice, however, the protein nitrogen accounted for only a small part of the total nitrogen present in the juice. In the low-nitrogen plants the protein nitrogen accounted for about 5 per cent. of the total nitrogen, in medium-nitrogen plants about 18 per cent., in high-nitrogen plants about 5 per cent., and in the control plants about 18 per cent. Moreover, the total-nitrogen content of juice from the high-nitrogen plants was higher than that from the medium-nitrogen plants, but the protein-nitrogen content was slightly lower.

The data in tables III and V show that there is a general correlation between virus concentration and nitrogen content in juice from diseased plants. In any one harvest, the juice with the highest virus concentration was always characterized by having the largest amount of both total and protein nitrogen. On the other hand, the juice with the lowest virus concentration had the smallest amount of nitrogen. These analyses offer definite evidence that the low virus activity in juice from low-nitrogen plants is due to a limitation in the nitrogen supply.

Discussion

It has commonly been supposed that the altered disease complex, brought about by variations in the nitrogen nutrition of the host, is due primarily to a change in the susceptibility of the host. Little attention has been given to the possibility that the causal agent of the disease may also be affected in some way by the mineral nutrition of the host. A review of the literature reveals several papers (**4, 15, 19, 20**) dealing with the effect of nitrogen on the severity of virus diseases, but no paper dealing with the effect of nitrogen on the concentration of the causative agent. Although such criteria as intensity of disease symptoms and retardation of growth show that host metabolism is altered during the course of the disease, they offer no evidence that the concentration of the causative agent is affected in any way.

The experimental data herein reported show that there are very definite variations in the concentration of the virus in juices of plants receiving different amounts of nitrogen. With our present knowledge, it is impossible

to say whether these variations are brought about directly by differences in the amounts of nitrogen supplied to the host or by changes in the physiological or morphological structure of the plant caused by these differences in nitrogen nutrition. It is obvious, however, that the ultimate virus concentration has been greatly affected in some way by the nitrogen supply. In view of these findings, the apparent increase in host susceptibility brought about by added nitrogen may be attributed, at least in part, to the increased content of virus present in these plants.

Competition between the normal growth processes and those responsible for virus increase may possibly be a factor in governing the extent to which virus is formed. When the supply is limited, most of the available nitrogen may be utilized for plant growth. As more and more nitrogen is made available, the supply of nitrogen above that needed for growth processes becomes greater, and this reservoir of nitrogen may account for the high virus content in plants on the high-nitrogen treatment. The nitrogen analyses show that plants on the high-nitrogen treatment contained a large amount of non-protein nitrogen that was presumably available for either plant growth or virus increase. With this large reservoir of nitrogen, it might be expected that the virus content in these plants would be higher than that demonstrated experimentally. Since protein content and virus activity are apparently closely associated, it is not improbable that the presence of some other element, such as phosphorus, in insufficient quantities may be a factor in limiting protein formation and also in limiting a further increase in the virus content of the high-nitrogen plants.

The experimental data show that the time required for the virus concentration to increase to a maximum in plant juice varied with the nitrogen supplied to the plant. This maximum was reached earlier with a low or medium supply of nitrogen than with an excess of nitrogen. Following this increase to a maximum, there appeared to be a decrease in virus content. The evidence indicates, therefore, that the supply of available nitrogen may control not only the increase but also the subsequent decrease in virus content. With a moderate supply of nitrogen, virus may increase more slowly than growth, resulting in an apparent decrease in virus concentration. With an excess of nitrogen, virus increase apparently continues unhampered but growth is retarded to some extent.

The experimental data show that juice from diseased plants had a much higher nitrogen content than that from corresponding healthy plants. These data are in agreement with those previously reported by other workers (**1, 2, 3, 5, 6, 11, 18**). The significance of this finding is difficult to evaluate with any degree of certainty. It is necessary to bear in mind that the nitrogen analyses recorded in table V are a measure of the soluble nitrogen in the juice after freezing, and not a measure of the total nitrogen either in the

plant as a whole or in freshly extracted juice. It is worthy of note, in this connection, that the difference between the total-nitrogen content in juices of healthy and diseased plants approximates very closely the difference in protein-nitrogen contents of the same juices. It is not improbable that some proteinaceous materials present in the juice of healthy plants may be relatively unstable and may become denatured by freezing and thereby rendered insoluble. This protein fraction would then be removed from the juice by the subsequent centrifugation used to clear the juice prior to analysis. Corroboration of this interpretation may be found in recent work of BAWDEN and PIRIE (3) who showed that a high molecular weight protein present in freshly extracted juice of healthy plants was converted irreversibly into an insoluble material when the juice was subjected to freezing.

Summary

The influence of nitrogen nutrition on virus production in Turkish tobacco plants was studied by supplying them with nutrient solutions containing either a low, medium, or high level of nitrogen, inoculating them with tobacco-mosaic virus, and measuring the virus activity in juice from frozen plants harvested at 3 different intervals following inoculation.

The virus activity of expressed juice was directly correlated with the amount of nitrogen supplied to the plants. The virus concentration of juice from plants receiving an ample nitrogen supply was over 80 times that of juice from nitrogen-deficient plants. Moreover, the virus content of expressed juice from plants in sand receiving the medium-nitrogen solution was more than double that found in juice from plants grown in composted soil. It is not improbable that the low virus activity of expressed juice from nitrogen-deficient plants is brought about by the limitation in nitrogen supply.

With insufficient nitrogen, the total virus content in extracted juice decreased following the initial systemic spread of the virus through the plant. With an over-abundance of nitrogen, the virus content increased at a faster rate than that at which the plants grew.

The increase in virus content showed no apparent correlation with the growth of the plants. Although the soil-grown plants were much larger than those in sand that received the high-nitrogen solution, they had only about $\frac{2}{3}$ as much total virus in their expressed juice.

The total-nitrogen and protein-nitrogen contents in the expressed juice varied directly with the nitrogen level of the nutrient solution. These amounts were much larger in juice from diseased plants than in juice from healthy plants on corresponding treatments. Virus activity was directly correlated with the nitrogen content in juice of diseased plants. The juice

with the highest total- and protein-nitrogen contents was found to have the highest virus activity.

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FLORAL DEVELOPMENT OF CERTAIN SPECIES AS INFLUENCED BY X-RADIATION OF BUDS

EDNA LOUISE JOHNSON

(WITH TWO FIGURES)

Introduction

X-radiation of susceptible plants which are nearing the reproductive stage usually causes abscission of buds or certain types of anomalies in flower structure. An earlier paper by the writer (3) reports the occurrence of abnormalities of floral parts including the production of double flowers when tomato plants were given three X-ray doses previous to blossoming. A single heavy dose usually causes bud abscission; later growth may produce blossoms some of which are normal, while others are double or triple.

GOODSPEED (1) noted that in *Nicotiana langsdorffi* buds of the terminal inflorescence were abscissed immediately after treatment and all the first flowers on laterals were abnormal. After a short time, only normal flowers were produced. MOORE and HASKINS (4) reported that irradiated petunia buds gave rise to blossoms displaying various anomalies.

The present article discusses various types of abnormalities in flower structure which result when young buds are irradiated with one medium dose. A rather extended analysis of the relationship found to exist between the degree of bud development at the time of treatment and the occurrence and character of the anomalies produced will be given for one of the species used in the experimentation.

Materials and methods

Plants of *Phlox drummondii, Nicotiana tabacum,* and *Salpiglossis sinuata* on which the young buds had developed were irradiated with one medium dose of X-rays; records were then taken of the dates of blooming together with a statement concerning the occurrence of either normal flowers or of those with floral abnormalities. If the latter were present, a brief description of each was given. The exact size of the buds of the first two species was not recorded at the time of irradiation. It seemed desirable, however, to use the third species, Salpiglossis, to determine whether there is a definite relationship between the degree of bud development and the type of abnormality produced. For this study, over 1500 blossoms from irradiated and control buds were used. The lengths of all buds one centimeter or over, and the number of all those under one centimeter, were recorded; these buds were then tagged and numbered as were also those developing later on the vegetative tissue which was not present at the time of treatment. Accurate records could thus be kept of the time of opening of each bud and of the character of the floral anomaly, if any appeared.

Experimentation

Phlox

Three groups of *Phlox drummondii* plants were given doses of 2000, 1800, and 1500 r-units respectively. Well-developed buds on plants receiving 2000 r-units survived and usually developed normally; the small buds which were present, abscissed. About the sixth week after treatment, blossoms developed from the new growth formed after irradiation of the plants. Floral anomalies frequently seen from the sixth to the eighth week included: (1) increase in number of corolla lobes; (2) occurrence of white streaks or spots on corolla lobes; and (3) a peculiar appearance of the corolla which has been referred to as "stippled" because the corolla looked as if it had been marked with tiny white dots. By the ninth week normal blossoms appeared in abundance, although some anomalies were evident up to the twelfth week when the experiment was concluded.

In the group receiving 1800 r-units, the corolla lobes of some flowers in every pot were either blotched or stippled two weeks after treatment. During the following four weeks, most of the plants produced no more flowers; between the seventh and eighth week, the maximum number of anomalies was evident. Throughout the entire experiment, the presence of dissected margins or fimbriated corolla lobes was the most frequently occurring anomaly, constituting one-third of the total number. A reproduced painting of one of the blossoms which developed on a plant 22 days after irradiation with 1800 r-units (plate IV, C–2) shows this character. Comparison with a control (plate IV, C–1) shows not only difference in the lobe margins but also reduction in size. Increased number of lobes and "stippled" corolla were also of common occurrence.

The dose of 1500 r-units given to the plants of group III caused abscission of only a few buds; and many of the blossoms developing during the first 3.5 weeks had anomalous characters. Flower production was decreased after this period, however; but later when blooming was more abundant, irregularities again appeared; corolla lobes with fimbriated edges and those with white streaks and spots were most prevalent.

Considering all groups as a whole, the anomaly occurring with the greatest frequency was that of the fimbriated or dissected corolla lobe. Others in order of occurrence were: increased number of lobes, appearance of white streaks or spots on the corolla lobes, color changes, decreased number of lobes, and dwarf blossoms (table I).

Nicotiana

A four-months record of floral development from budded *Nicotiana tabacum* plants which were exposed to one X-ray dose of 2000 r-units is given in table II. Some type of abnormality was evident in 57.5 per cent.

TABLE I

FLORAL ANOMALIES OF PHLOX PLANTS IRRADIATED AFTER BUD DEVELOPMENT

ANOMALIES	GROUP I 2000 R-UNITS	GROUP II 1800 R-UNITS	GROUP III 1500 R-UNITS	TOTAL OF ALL GROUPS
	%	%	%	%
Dissected or fimbriated corolla lobes	15.1	33.7	53.7	30.0
Increased number of lobes	35.8	30.0	9.2	27.9
White streaks or spots	19.8	6.2	18.5	15.0
White "stippling" on lobes	17.9	15.3	7.4	14.6
Color change in lobes	6.6	10.0	11.1	8.7
Decreased number of lobes	.9	5.0	0.0	2.1
Dwarf blossoms	3.8	0.0	0.0	1.7

of the blossoms from irradiated plants; the greatest number of these occurred during the first 20 days after irradiation and again in the period extending from 41 to 60 days. In general it was found that buds which were large enough to show color at the time of irradiation developed normally; those which were very small would either abscise or, after unfolding, show some anomaly. After the first 20 days, flowering was decreased until blossoms formed from buds which developed on new tissue appearing after treatment. Such blossoms which opened about the 40th day, showed the maximum number of irregularities; hence the largest number of anomalies is listed for the 41- to 60-day period. Normal blossoms began to appear in greater numbers in the 61- to 80-day period which was toward the end of the blossoming cycle, the average length of which was 65.7 days. The total flowering period for the different plants varied greatly, for in some cases scarcely any flowers developed after irradiation; in two-thirds of the irradiated plants, however, after abscission of buds borne on the original main stem, new branches formed which continued to bear blossoms.

About 30 per cent. of the anomalies are listed as "color change," the term being used to designate either a uniform lighter color which was characteristic of the entire flower or to indicate a light sector in one of the lobes (plate IV, A–4, 5). These color changes were evident throughout the entire experimental period, occurring with the greatest frequency at the time when the greatest number of anomalies was evident. Many of the blossoms which opened during the first 20 days showed the stippled appearance which was more pronounced than that shown in the Phlox (plate IV, A–2). In some cases these tiny white dots developed into streaks; in a considerable number of instances, however, prominent white streaks or spots were evident when the blossoms first unfolded (plate IV, A–3, 4). Stippling, spotting, and streaking were most common from the 21st to 60th day. GOODSPEED (2) has

TABLE II

FLORAL DEVELOPMENT OF NICOTIANA PLANTS

	PLANTS EXPOSED TO 2000 R-UNITS						
	1–20 DAYS	21–40 DAYS	41–60 DAYS	61–80 DAYS	81–120 DAYS	TOTAL NO.	PERCENTAGE
A. ANOMALIES OCCURRING IN BLOSSOMS FROM PLANTS IRRADIATED AFTER DEVELOPMENT OF BUDS							
Color change	3	1	8	4	7	23	29.48
Streaked	1	5	5	1	0	12	15.38
Stippled	9	2	0	0	0	11	14.10
Lobes unlike in size	6	1	2	0	0	9	11.53
Spotted	3	4	0	0	0	7	8.97
Tube split	1	0	4	1	0	6	7.7
Dwarf	1	2	1	1	0	5	6.41
Lobes increased in number	0	0	4	0	0	4	5.12
Lobes decreased in number	0	0	1	0	0	1	1.28
Total number	24	15	25	7	7	78	
Percentage of total	30.8	19.2	32.0	9.0	9.0		
B. NORMAL BLOSSOMS FROM PLANTS IRRADIATED AFTER DEVELOPMENT OF BUDS							
Total number	6	3	13	20	9	51	
Percentage of total	11.8	5.9	25.5	39.2	17.6		
CONTROLS (BLOSSOMS ALL NORMAL)							
Total number	21	22	20	7	4	74	
Percentage	28.4	29.7	27.0	9.4	5.4		

figured tobacco flowers with white dots and lines on the corolla lobes and speaks of them as representing areas of air-filled cells.

Variations in number and size of corolla lobes were numerous. Permanent decreased size of upper lobes was marked in some specimens. Increase in corolla-lobe number (plate IV, A–6) over that of the normal was responsible for five per cent. of the anomalies while but one per cent. showed a reduced number of lobes. Cases of split corolla tube, often accompanied by abnormal curvature and marked dwarfness, were other unusual features in the blossoms of irradiated plants.

The production of blossoms by controls was rather evenly distributed over the first 60 days. All of the flowers were normal except two which showed a few white spots.

Salpiglossis

Salpiglossis sinuata, the third species which was treated in the budded stage, was also given 2000 r-units. At the time of irradiation, the lengths of all buds one centimeter or over in length and the number of those under one centimeter were recorded. A numbered tag was attached to each bud; when the blossom opened, records were made of the date and appearance of each flower.

Over 1500 blossoms from irradiated buds and controls were thus observed over a period of three months. Results are given for 991 blossoms on the irradiated plants and 453 on the controls. Cases were not included where records were incomplete as to the size of buds at time of irradiation. Two plants were frequently grown in one pot; when one had buds of the size desired for radiation, the second was often small with few buds visible. The plants which were more mature at the time of irradiation were placed in group I while those which were less well developed were considered as group II. Though the types of anomalies produced were practically the same in both cases, it seemed best to record the results separately, as plants in group II were often slightly less vigorous than those of the first group.

Of the 741 blossoms from the irradiated plants of group I, 25 per cent. were normal; the remainder exhibited 770 anomalies of ten different types (table III). The 20-day period in which the blossoms opened, and the bud size at the time of irradiation, have been considered in the tabulation. The anomalies are listed according to their frequency of occurrence and will be discussed in that order. A spotted condition of the corolla constituted 24.5 per cent. of all the abnormalities (plate IV, B–2, 5); in many cases, the spots which were very small and close together gave the appearance of stippling. Normally the corolla is of a velvety texture, but the occurrence of the dots

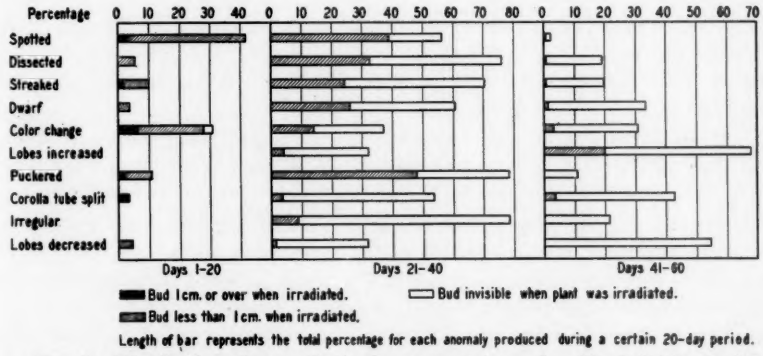


FIG. 1. Distribution of anomalies in flower structure produced by Salpiglossis plants exposed to X-radiation.

TABLE III

PERCENTAGE OF ANOMALIES FOUND AMONG 741 FLOWERS PRODUCED BY 46 SALPIGLOSSIS PLANTS WHICH WERE EXPOSED TO 2500 R-UNITS OF X-RAYS (GROUP I)

TYPE OF FLORAL ANOMALY	DAYS 1–20 SIZE OF BUD WHEN IRRADIATED				DAYS 21–40 SIZE OF BUD WHEN IRRADIATED				DAYS 41–60 SIZE OF BUD WHEN IRRADIATED				DAYS 61–80 BUDS ALL INVISIBLE TOTAL	TOTAL PER CENT. OF ALL ANOMALIES
	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVISIBLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVISIBLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVISIBLE		
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Spotted	41.8	6.3	93.7	0.0	56.1	0.0	69.8	30.2	2.1	0.0	25.0	75.0	0.0	24.5
Dissected	5.3	0.0	100.0	0.0	75.8	0.0	43.0	57.0	18.9	0.0	4.0	96.0	0.0	17.1
Streaked	9.9	15.4	84.6	0.0	70.2	0.0	34.8	65.2	19.8	0.0	3.8	96.2	0.0	17.0
Dwarf	3.6	0.0	100.0	0.0	60.7	0.0	43.1	56.9	33.3	0.0	3.8	96.2	2.4	10.9
Color change	30.8	20.0	70.0	10.0	36.9	0.0	37.5	62.5	30.8	0.0	10.0	90.0	1.5	8.4
Lobes increased	0.0				32.0	0.0	12.5	87.5	68.0	0.0	29.4	70.6	0.0	6.5
Puckered	10.9	20.0	80.0	0.0	78.3	0.0	61.1	38.9	10.9	0.0	0.0	100.0	0.0	6.0
Corolla tube split	3.6	100.0	0.0	0.0	53.6	0.0	6.7	93.3	42.9	0.0	8.3	91.7	0.0	3.6
Irregular	0.0				78.3	0.0	11.1	89.9	21.7	0.0	0.0	100.0	0.0	3.0
Lobes decreased	4.5	0.0	100.0	0.0	31.8	0.0	4.5	95.5	54.5	0.0	0.0	100.0	9.0	2.8

obscured this appearance and gave it a "frosty" look. A study of table III and figure 1 indicates that this spotted condition was very prevalent during the first 40 days and that it was a characteristic response in flowers from buds which were less than 1 cm. in length at the time of radiation. The dissected, ragged, or fringed appearance of the corolla lobes apparent in plate IV, B–5, 6 was especially common among flowers opening during the 21- to 40-day period. The greater number appearing in from 21 to 60 days were from buds which were invisible at the time of radiation (fig. 1).

Definite white or yellowish streaks on corolla tubes occurred most frequently in the 21- to 40-day period (fig. 1); the higher percentage in this period as well as in the later one was from buds which were invisible at the time of treatment. It may be noted that the percentage of streaked corollas occurring in the 1- to 20-day period was considerably less than the spotted ones. MOORE and HASKINS (**4**) found that Petunia buds irradiated about 16 days before the blossoms opened showed corollas with white stippling. In turn, there followed flowers with coarsely flecked corollas and others exhibiting white lines and wedges. Flowers developing later were normal. Petunia, however, was not reported as having many of the other morphological variations exhibited by flowers of the irradiated Salpiglossis plants.

Few dwarf blossoms occurred in the first 20-day period, the higher percentage being present in the 21- to 40-day period. The plants which continued their flowering for as long as 80 days, developed dwarf blossoms which came from buds which were invisible at the time of treatment.

The term "color change" is used to include conspicuous grayish margins apparent in some specimens, as well as changes in the color of certain lobes (plate IV, B–3). These color changes were about equally distributed between the first three 20-day periods. A few gray-margined blossoms were also evident among the control plants of this group.

In but two types of anomalies, increased and decreased number of corolla lobes, was the highest percentage of occurrence found in the 41- to 60-day period. The percentage of corollas with an abnormally large number of lobes was higher than for those with a decreased number; the latter irregularity was the most frequent one found during the 61- to 80-day period.

A "puckered" appearance of the corolla tissue similar to that seen in crepe cloth constituted 6 per cent. of all the anomalies of group I. This was a new type of anomaly not present in the Phlox or tobacco, but it has been reported by MORGAN (**5**) as occurring in blossoms developing from irradiated Freesia bulbs. The greater number of these puckered corollas occurred in the 21- to 40-day period, and the highest percentage came from buds less than 1 cm. in length at time of irradiation. Cases of split corolla tube occurred in Salpiglossis as well as in Nicotiana. They were more in evidence from the 21st to the 60th day; by far the greater number were from buds invisible at the time of irradiation (fig. 1).

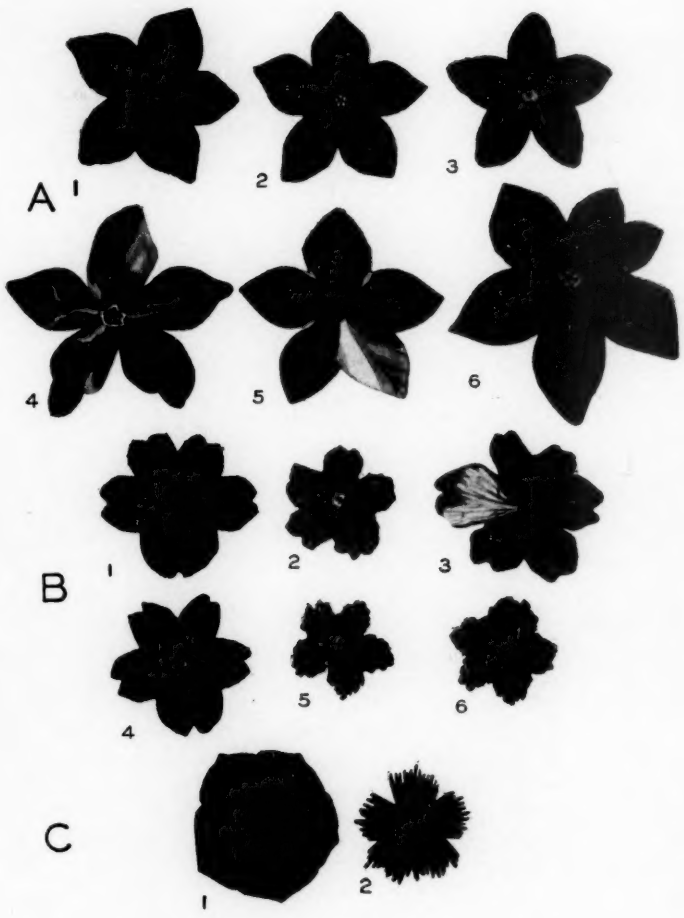
Explanation of Plate IV

Blossoms of plants which were X-radiated after development of buds

A. Blossoms of *Nicotiana tabacum* from plants which were producing buds when X-radiated with a dose of 2000 r-units. 1, control; 2, blossom which opened 18 days after bud had received 2000 r-units; note stippled appearance of corolla lobes; 3, blossom which opened in 33 days from bud which was invisible at time of treatment; 4, blossom (×2.5) which appeared on a secondary branch formed after treatment; light streaks and sectors are conspicuous; 5, blossom with absence of color in one lobe of corolla which opened 60 days after treatment; 6, blossom (×2.5) with deeply divided lobe of corolla which opened 54 days after treatment; branch on which it grew had not formed at time of irradiation.

B. *Salpiglossis sinuata* blossoms from plants which were producing buds when irradiated with a dose of 2000 r-units. 1, control; 2, dwarf blossom with spotted, puckered tissue which opened 35 days after treatment; 3, blossom with increase in lobe number as well as change in color; blossom opened 48 days after treatment; 4, control; 5, dwarf blossom with spotted, puckered tissue which opened 39 days after treatment; the bud at time of irradiation was invisible; 6, blossom which opened 26 days after treatment from bud under 1 cm. in length when irradiated.

C. *Phlox drummondii*. 1, control (×2.5); 2, blossom (×3) with conspicuous fimbriated lobes which developed on plant 22 days after irradiation with 1800 r-units. Many cases of this anomaly occurred.



Johnson: X-radiation

TABLE IV

TYPES OF ANOMALIES FOUND AMONG THE 250 FLOWERS PRODUCED BY 16 IRRADIATED SALPIGLOSSIS PLANTS WHICH WERE EXPOSED TO 2500 R-UNITS (GROUP II)

TYPE OF FLORAL ANOMALY	DAYS 1–20 SIZE OF BUD WHEN IRRADIATED				DAYS 21–40 SIZE OF BUD WHEN IRRADIATED				DAYS 41–60 BUDS ALL INVISIBLE TOTAL	TOTAL PER CENT. OF ALL ANOMALIES
	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVISIBLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVISIBLE		
	%	%	%	%	%	%	%	%	%	%
Spotted	51.6	10	90	0.0	46.9	0.0	40	60	1.6	25.6
Streaked	8.8	0.0	100	0.0	84.2	0.0	31.3	68.7	7.0	22.8
Color change	26.7	37.5	62.5	0.0	26.7	0.0	37.5	62.5	46.7	12.0
Dwarf	7.1	0.0	100	0.0	53.6	0.0	0.0	100.0	39.3	11.2
Dissected	3.6	0.0	100.0	0.0	89.3	0.0	8.0	92.0	7.1	11.2
Irregular	13.3	100.0	0.0	0.0	33.3	0.0	20.0	80.0	53.3	6.0
Lobes increased	0.0	0.0	0.0	0.0	72.7	0.0	0.0	100.0	27.3	4.4
Puckered	0.0	0.0	0.0	0.0	87.5	0.0	28.6	71.4	12.5	3.2
Lobes decreased	0.0	0.0	0.0	0.0	80.0	0.0	0.0	100.0	20.0	2.0
Corolla tube split	0.0	0.0	0.0	0.0	75	0.0	0.0	100.0	25.0	1.6

Cases listed as "irregular" refer to an irregularity in corolla lobes such as the appearance of a small lobe inside a large one, or a lobe abnormal in shape and size.

In the irradiated plants of group I and in the controls (table VI) the greater number of blossoms as well as the greater number of anomalies developed during the 21- to 40-day period; the smallest number in the 61- to 80-day period. The others were rather evenly distributed in the first and third periods.

Anomalous floral structures similar to those just discussed were also evident in the blossoms of group II. These have been classified and listed in table IV. It may be noted that there is but slight difference in the order of frequency of the occurrence of the anomalies for the two groups; thus results for the second group serve to confirm the conclusions drawn for the first. The summarized record of the two groups as shown in table V indicates that only 25 per cent. of the blossoms in the first group and 21 per cent. in the second group were normal. All others showed at least one type of abnormality. In both groups the greatest number, approximately 60 per cent. in each, were evident in the 21- to 40-day period. The highest percentage in both groups during this period was from the lot in which the buds at time of irradiation were invisible. The percentage of anomalies in the 1- to 20-day period and those in the 41- to 60-day period were practically the same in both groups. In the first period, blossoms from irradiated buds under 1 cm. in length had the highest percentage of anomalies; in the 41- to 60-day period, the group in which the buds were invisible at radiation contained the highest percentage.

One noticeable difference between the two groups is the fact that the irradiated plants of group I, which produced normal blossoms, bore the greater proportion of them during the first 20 days and from buds which were over 1 cm. in length at the time of treatment. Of the normals produced by irradiated plants of group II, the greater number were borne in the 21- to 40-day period and the highest percentage came from buds less than 1 cm. in length at time of treatment.

Blossoms from the 39 control plants numbered 454. Among the first group 10 per cent. showed anomalous characters, the chief of which was color change (table VI). Among plants of the second group, 13 per cent. gave evidence of some irregularities, chief of which were color changes and irregular corollas. The greater number of blossoms in the control groups appeared in the 21- to 40-day period.

Summary

Blossoms developing from plants of *Salpiglossis sinuata, Phlox drummondii,* and *Nicotiana tabacum* which had been irradiated during the repro-

TABLE V

SUMMARIZED RECORD OF BLOSSOMS FROM IRRADIATED SALPIGLOSSIS PLANTS

	Days 1–20 Size of bud when irradiated				Days 21–40 Size of bud when irradiated				Days 41–60 Size of bud when irradiated				Days 61–80 Buds all invisible
	Total	1 cm. or over	Less than 1 cm.	Invisible	Total	1 cm. or over	Less than 1 cm.	Invisible	Total	1 cm. or over	Less than 1 cm.	Invisible	Total
Group I. Record of 741 blossoms from forty-six plants													
Anomalies in 555 blossoms													
Number	129	13	114	2	465	0	210	255	171	0	8	163	5
Percentage	10.7	10.0	88.4	1.5	60.4	0.0	45.2	54.8	22.2	0.0	4.7	95.3	6.5
Normal blossoms (25.1 per cent. of total)													
Number	80	53	27	0	37	0	11	26	65	0	3	62	4
Percentage	43.0	66.3	33.7	0.0	19.9	0.0	29.7	70.3	34.9	0.0	4.6	95.4	2.1
Group II. Record of 250 blossoms from sixteen plants													
Anomalies in 197 blossoms													
Number	51	8	43	0	153	0	35	118	46	0	0	46	
Percentage	20.4	15.7	84.3	0.0	61.2	0.0	22.9	77.1	18.4	0.0	0.0	100.0	
Normal blossoms (21.2 per cent. of total)													
Number	15	8	7	0	26	0	20	6	12	0	0	12	
Percentage	28.3	53.3	46.7	0.0	49.0	0.0	76.9	23.1	22.6	0.0	0.0	100.0	

TABLE VI

RECORD FOR 454 BLOSSOMS FROM 39 CONTROL SALPIGLOSSIS PLANTS

	TOTAL	DAYS 1–20	DAYS 21–40	DAYS 41–60	DAYS 61–80
GROUP I (327 BLOSSOMS FROM 26 PLANTS)					
Number of normal blossoms	292	100	127	61	4
Percentage of total	89.3	34.2	43.5	20.9	1.4
Number of anomalies	35	0	14	20	1
Percentage of total	10.7	0	40	57.1	2.9
GROUP II (127 BLOSSOMS FROM 13 PLANTS)					
Number of normal blossoms	110	9	61	40	
Percentage of total	86.6	8.2	55.4	36.4	
Number of anomalies	17	0	14	3	
Percentage of total	13.4	0	82.3	17.6	

ductive stage with one medium dose of X-rays showed approximately 10 types of floral anomalies. The following were common to all three species: white stippling, spotting, and streaking of corolla lobes, color changes, increased and decreased number of corolla lobes, and dwarf blossoms. *Salpiglossis sinuata* was the only species of the three having puckered corolla tissue and irregularity in placement of floral organs. Cases of split corolla tube evident in Nicotiana and Salpiglossis were not seen in Phlox. Dissected or fimbriated margins of corolla lobes so conspicuous in Phlox and Salpiglossis were absent in Nicotiana.

Buds of Phlox and Nicotiana were markedly ray-susceptible, for a dose of 2,000 r-units caused frequent abscission of buds in these two species; but the same dose did not cause bud abscission in Salpiglossis.

A definite relationship was found between the size of the buds at the time of irradiation and the occurrence and character of the anomaly produced in the flower. From the study with Salpiglossis in which hundreds of buds were measured at the time of radiation, it was definitely determined that buds over one centimeter in length at the time of irradiation usually developed normally. Many of those under one centimeter in length exhibited various types of irregularities as did those forming from new growth which developed after the plants were treated. Types of anomalies generally appearing during the early part of the blooming period included stippling, spotting, and streaking of the corolla, also changes in color. Those which more commonly occurred during the middle and latter part of the flowering period were split corolla tubes, dissected margin of corolla lobes, puckered corolla tissue, increase and decrease in number of corolla lobes, and dwarf blossoms. Toward the end of the blooming period, normal blossoms began to appear in increased numbers.

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SOME EFFECTS OF METAL SALTS ON YEAST DEHYDROGENASE

CHARLES WILLIAM HOCK

(WITH ONE FIGURE)

The results of many investigations show that solutions of electrolytes have pronounced effects upon biological systems. Permeability, viscosity, elasticity, irritability, respiration, and growth are but a few of the properties of such systems which are influenced by the presence of various anions and cations. It is now generally recognized that dehydrogenases, *i.e.*, respiratory enzymes concerned with the activation and the removal of hydrogen from the substrates which become oxidized, are universally distributed in plant and animal cells, and that they play an important rôle in normal respiration (**4, 5, 6, 9**). The present investigation was undertaken to determine the effects of certain representative metals on the dehydrogenase systems of yeast and of macerated yeast extract.

Materials and method

Bakers' yeast (or extract thereof) was used for all experiments. In order to reduce to a minimum traces of heavy metals which if present might influence the reactions, all solutions were made with water which had been twice distilled. For buffering these solutions phosphates could not be used, as most of the metals under investigation form precipitates with the phosphate ion. Accordingly, a sodium acetate-acetic acid buffer mixture maintaining a pH of 6.2 was selected (**11**). This was made by adding one part of 0.1 M acetic acid to ten parts of 0.5 M sodium acetate.

SUSPENSION OF YEAST CELLS

The suspension was made by mixing one part of yeast with two parts of buffer. This was done just before each trial. Tests showed that the buffer mixture was efficient in maintaining a constant pH of 6.2 throughout the course of an experiment.

MACERATED YEAST EXTRACT

Equal amounts, by weight, of yeast and of powdered glass were ground in a mortar until a thick paste was obtained. In preliminary tests the glass powder was washed several times with distilled water to remove any soluble impurities which might influence the reaction. Since no differences could be observed between extracts prepared with washed and with unwashed glass, the practice was discontinued. There was added to the ground yeast paste twice as much buffer as yeast contained therein. This made the

amount of yeast in one volume of extract equal to that in the same volume of whole yeast suspension. After a thorough stirring the mixture was filtered through Whatman no. 1 filter paper and the extract thus obtained was kept in a cool place until needed. Since it was noted that the yeast

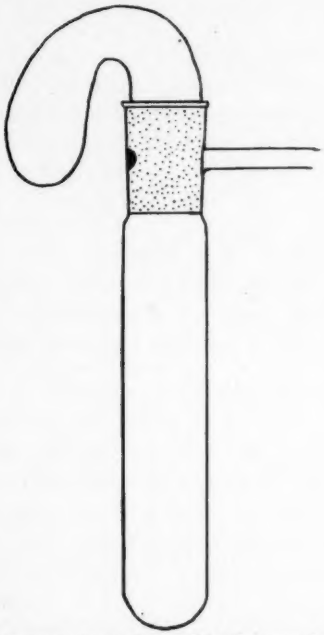

FIG. 1. THUNBERG test-tube used in the experiments reported.

extract lost some of its dehydrogenase potency upon standing, every effort was made to use the extract as soon as possible.

CHEMICAL REAGENTS

Solutions of the following metal salts were used in the tests: KCl, KNO_3, $CuCl_2 \cdot 2H_2O$, $HgCl_2$, $MnCl_2 \cdot 4H_2O$, $BaCl_2 \cdot 2H_2O$, $AuCl_3 \cdot 2H_2O$, $La(NO_3)_3 \cdot 6H_2O$, $Th(NO_3)_4 \cdot 4H_2O$. In every case the purest salts obtainable were employed. A 1:5000 dilution of methylene blue was used as the indicator, and sodium lactate in a final concentration of 0.02 N was added as a substrate.

METHOD

The simple and widely used THUNBERG technique was employed. That form of the THUNBERG test-tube with the hollow stopper (fig. 1) was selected because of its adaptability to the present investigation.

When a solution of methylene blue is added to a suspension of yeast cells in the presence of a substrate such as sodium lactate, and the whole system evacuated, a rapid reduction to colorless methylene blue results, while the lactic acid is oxidized. In such cases the dye acts as an acceptor for the two hydrogen atoms removed from the substrate. The plan adopted was to add increasing dilutions of salts to such systems and to note the effects of the metals on the rate of decolorization.

Both the yeast suspension and the yeast extract will decolorize methylene blue without the addition of a substrate, owing to the presence of oxidizable substrates normally present in the yeast itself, but the rate of the reaction is greatly accelerated by the addition of more substrate such as sodium lactate, here employed in a final concentration of 0.02 N. In all experiments each tube contained 1 ml. of methylene blue, 1 ml. 0.02 N sodium lactate, and 1 ml. of the metal salt solution. Two ml. of the yeast suspension or of the yeast extract was put in the hollow stopper. The tubes were then evacuated and allowed to come to 30° C. in a constant temperature chamber. After mixing, the time of decolorization in each case was noted. A control was run in which distilled water was substituted for the salt solution.

Results

Table I records the time of decolorization by suspensions of yeast cells in the presence of various concentrations of the metal salts. Table III gives the results when macerated yeast extract was used. Tables II and IV record for yeast suspensions and for yeast extract, respectively, the percentage of increase or decrease in time of decolorization caused by the addition of the metals to the systems. By reducing the results of all tests to a percentage basis, individual differences among control tubes are eliminated, and the results for yeast suspensions and for yeast extract are comparable. Each reading in the tables represents an average of ten tests. Since chlorides of some metals and nitrates of others were used, solubility usually being the deciding factor, it became necessary to see whether or not these anions were significantly different in behavior. Since tests showed such differences to be negligible, we may conclude that variations in acceleration and in inhibition are due to the cations.

Potassium chloride and potassium nitrate

Since only slight differences were noted between KCl and KNO_3 the averages for the two have been taken. All concentrations which were tried caused some inhibition when used with yeast cells, although of all the cations examined, this was the least inhibitive. At all except relatively high concentrations the inhibition was slight. When used with yeast extract the situation was somewhat different. High concentrations, from 0.2 N to

TABLE I

METAL SALTS ADDED TO SUSPENSIONS OF YEAST CELLS

CONCENTRATION OF SALT IN SYSTEM	TIME OF DECOLORIZATION RECORDED IN MINUTES							
	KCl KNO_3	$CuCl_2$	$HgCl_2$	$MnCl_2$	$BaCl_2$	$AuCl_3$	$La(NO_3)_3$	$Th(NO_3)_4$
Normality	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
0.4 N					26.2			
0.2 N	17.1	*	*	35.0	24.2	*		*
2.0×10^{-1} N	13.8	*	*	20.3	18.0	220.0	29.3	83.8
4.0×10^{-2} N		262.0	421.0	17.9	13.8	16.3	20.0	42.8
2.0×10^{-2} N	13.0	121.0	108.0	14.5	13.1	11.2	14.8	16.5
6.6×10^{-3} N		86.2	79.9			9.2	11.0	10.4
4.0×10^{-3} N		53.5	59.0	14.2	12.0	8.1	11.3	9.8
2.0×10^{-3} N	12.8	32.3	40.4	13.0	12.7	8.4	11.7	10.1
4.0×10^{-4} N	13.2	14.0	29.2					
2.0×10^{-4} N	13.1	13.3	17.4	12.9	12.0	8.3	11.4	10.6
2.0×10^{-5} N	12.4	13.6	16.8	12.6	12.8	8.8	11.2	10.4
2.0×16^{-6} N	12.9	13.8	17.1	12.4	11.6	8.6	10.8	10.6
2.0×10^{-7} N	12.6	14.0		12.4	10.9	8.8	10.6	10.6
2.0×10^{-8} N		14.3		12.4			10.6	
2.0×10^{-9} N		14.5		12.6			10.4	
2.0×10^{-10} N		15.3		12.2				
2.0×10^{-11} N		14.7		12.6				
2.0×10^{-12} N		14.7		13.1				
2.0×10^{-13} N		14.7		13.1				
Control	12.5	14.5	16.7	13.1	10.9	8.6	10.4	10.6

* No decolorization in 12 hours.

TABLE II

METAL SALTS ADDED TO SUSPENSIONS OF YEAST CELLS

CONCENTRATION OF SALT IN SYSTEM	PERCENTAGE INCREASE OR DECREASE IN TIME OF DECOLORIZATION CAUSED BY METALS							
	KCl KNO_3	$CuCl_2$	$HgCl_2$	$MnCl_2$	$BaCl_2$	$AuCl_3$	$La(NO_3)_3$	$Th(NO_3)_4$
Normality	%	%	%	%	%	%	%	%
0.4 N					+140.0			
0.2 N	+37.0	*	*	+160.0	+120.0	*		*
2.0×10^{-1} N	+10.0	*	*	+ 55.0	+ 65.0	+2400.0	+180.0	+690.0
4.0×10^{-2} N		+1700.0	+2400.0	+ 35.0	+ 25.0	+45.0	+ 90.0	+300.0
2.0×10^{-2} N	+ 4.0	+ 730.0	+ 550.0	+ 10.0	+ 20.0	+30.0	+ 40.0	+ 55.0
6.6×10^{-3} N		+ 490.0	+ 380.0			+ 6.9	+ 5.7	- 1.9
4.0×10^{-3} N		+ 270.0	+ 250.0	+ 8.4	+ 10.0	- 5.8	+ 8.6	- 7.5
2.0×10^{-3} N	+ 4.8	+ 120.0	+ 140.0	- 0.7	+ 15.0	- 2.1	+ 10.0	- 4.7
4.0×10^{-4} N		- 3.4	+75.0					
2.0×10^{-4} N	+ 2.4	- 8.2	+ 4.1	- 1.5	+ 10.0	- 3.4	+ 9.6	0.0
2.0×10^{-5} N	+ 5.6	- 6.2	+ 0.6	- 3.8	+ 15.0	+ 2.1	+ 7.7	- 1.9
2.0×10^{-6} N	+ 4.8	- 4.1	+ 0.6	- 5.3	+ 6.4	0.0	+ 3.8	0.0
2.0×10^{-7} N	- 0.8	- 3.4	+ 2.4	- 5.3	0.0	+ 2.1	+ 1.9	0.0
2.0×10^{-8} N	+ 3.2	- 1.3		- 5.3			+ 1.9	
2.0×10^{-9} N	+ 0.8	0.0		- 3.8			0.0	
2.0×10^{-10} N		+5.5		- 6.8				
2.0×10^{-11} N		+1.3		- 3.8				
2.0×10^{-12} N		+1.3		0.0				
2.0×10^{-13} N		+1.3		0.0				

* No decolorization in 12 hours.

TABLE III

METAL SALTS ADDED TO MACERATED YEAST EXTRACT

CONCENTRATION OF SALT IN SYSTEM	TIME OF DECOLORIZATION RECORDED IN MINUTES							
	KCl KNO_3	$CuCl_2$	$HgCl_2$	$MnCl_2$	$BaCl_2$	$AuCl_3$	$La(NO_3)_3$	$Th(NO_3)_4$
Normality	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
0.4 N					1.5			
0.2 N	2.2	*	*	1.4	1.7	*		*
2.0×10^{-1} N	2.4	*	*	1.5	2.1	48.0	5.5	10.4
4.0×10^{-2} N		535.0	*	2.5	2.4	4.4	4.1	3.3
2.0×10^{-2} N	2.5	250.0	244.0	2.7	2.8	4.3	2.7	3.2
6.6×10^{-3} N		56.4	231.0			3.4	2.8	3.5
4.0×10^{-3} N		19.8	101.0	3.0	2.8	3.2	2.8	4.2
2.0×10^{-3} N	2.6	7.7	56.9	2.9	2.9	3.9	2.7	4.3
4.0×10^{-4} N		6.1	8.4					
2.0×10^{-4} N	2.7	5.4	5.6	2.9	2.7	3.8	3.0	4.4
2.0×10^{-5} N	2.8	5.4	4.3	2.9	2.6	3.9	2.7	4.4
2.0×10^{-6} N	2.7	5.5	4.2	2.6	2.7	3.8	2.5	4.3
2.0×10^{-7} N	2.8	5.2	4.4	2.7	2.5	3.9	2.7	4.5
2.0×10^{-8} N	2.8	5.8	4.0	2.8			2.4	
2.0×10^{-9} N	2.7	5.7	3.8	2.6			2.4	
2.0×10^{-10} N		5.7	4.6	2.6				
2.0×10^{-11} N		5.8		2.6				
2.0×10^{-12} N		6.0		2.6				
2.0×10^{-13} N		5.7		2.9				
Control	2.7	5.7	4.6	2.9	2.5	3.5	2.5	4.2

* No decolorization in 12 hours.

TABLE IV

METAL SALTS ADDED TO MACERATED YEAST EXTRACT

CONCENTRATION OF SALT IN SYSTEM	PERCENTAGE INCREASE OR DECREASE IN TIME OF DECOLORIZATION CAUSED BY METALS							
	KCl KNO_3	$CuCl_2$	$HgCl_2$	$MnCl_2$	$BaCl_2$	$AuCl_3$	$La(NO_3)_3$	$Th(NO_3)_4$
Normality	%	%	%	%	%	%	%	%
0.4 N					−40.0			
0.2 N	−19.0	*	*	−52.0	−32.0	*		*
2.0×10^{-1} N	−11.0	*	*	−48.0	−16.0	+1300.0	+64.0	+150.0
4.0×10^{-2} N		+9300.0	*	−14.0	− 4.0	+ 25.0	+ 8.0	− 20.0
2.0×10^{-2} N	− 7.4	+4300.0	+5200.0	− 6.9	+12.0	+ 23.0	+12.0	− 23.0
6.6×10^{-3} N		+ 890.0	+4900.0			−2.8	+12.0	− 16.0
4.0×10^{-3} N		+ 250.0	+2900.0	(?) + 3.4	+12.0	−8.5	+ 8.0	0.0
2.0×10^{-3} N	− 3.7	+ 35.0	+1100.0	(?) 0.0	+16.0	+ 11.5	+ 8.0	+ 2.3
4.0×10^{-4} N		+7.4	+85.0		+ 8.0			
2.0×10^{-4} N	0.0	−2.1	+20.0	(?) 0.0	+ 4.0	+8.6	+20.0	+ 4.7
2.0×10^{-5} N	+ 3.7	−2.1	− 6.5	(?) 0.0	+ 8.0	+ 11.0	+ 8.0	+ 4.7
2.0×10^{-6} N	0.0	−1.3	− 8.7	−10.0	0.0	+8.6	0.0	+ 2.3
2.0×10^{-7} N	+ 3.7	−8.7	− 4.3	− 6.9		+ 11.0	+ 8.0	+ 7.1
2.0×10^{-8} N	+ 3.7	+1.7	+13.0	− 3.4			0.0	
2.0×10^{-9} N	0.0	0.0	+17.0	−10.0				
2.0×10^{-10} N		0.0	0.0	−10.0				
2.0×10^{-11} N		+1.7		−10.0				
2.0×10^{-12} N		+2.1		−10.0				
2.0×10^{-13} N		0.0		0.0				

* No decolorization in 12 hours.

2.0×10^{-3} N, increased the velocity of decolorization. Lower concentrations were without effect or very slightly inhibitive. FLEISCHMANN and SCHWARZ (**2**), using yeast cells, found that sodium accelerated the dehydrogenation of sodium succinate whereas potassium inhibited the oxidation. They observed no differences between these two ions when added to macerated yeast extract.

CUPRIC CHLORIDE

When $CuCl_2$ is present in a concentration of 0.02 N or greater the dehydrogenase activity of yeast cells and of extract is completely inhibited. In concentrations ranging from 4.0×10^{-4} N to 2.0×10^{-8} N, inclusive, the rate of decolorization of methylene blue by yeast cells is increased; concentrations from 2.0×10^{-4} to 2.0×10^{-7} N have a slightly accelerating effect when yeast extract is used.

MERCURIC CHLORIDE

All concentrations of this salt exerted an inhibitive action on a suspension of yeast cells. No decolorization by yeast cells was obtained when concentrations 0.02 N or greater were used. The mercuric ion accelerated the dehydrogenase activity of extract when present in concentrations from 2.0×10^{-5} N to 2.0×10^{-9} N, inclusive. Concentrations of greater normality were inhibitive.

MANGANESE CHLORIDE

Down to a concentration of 4.0×10^{-3} N this cation exerted inhibitive action on suspensions of yeast cells. At greater dilutions, as great as 2.0×10^{-11} N, the presence of the metal hastened decolorization of the dye, *i.e.*, the metal promoted dehydrogenase activity. The effect of this metal on yeast extract was remarkable. All concentrations from 0.2 N to 2.0×10^{-12} N seemed able to reduce the time of decolorization.

BARIUM CHLORIDE

Concentrations ranging from 0.4 N to 2.0×10^{-6} N retarded slightly the decolorization of methylene blue by the dehydrogenase of yeast cells. Low concentrations of the metal were weakly toxic to yeast extract. The dehydrogenation of lactic acid by extract was definitely accelerated by higher concentrations of $BaCl_2$, as much as 40 per cent. when barium was present in a 0.4 N concentration. Although barium exerted a remarkable promoter action, this was evident only in high concentrations—4.0×10^{-2} N and greater.

AURIC CHLORIDE

At concentrations between 4.0×10^{-3} N and 2.0×10^{-4} N, inclusive, the gold ion increased the velocity of reaction with suspensions of yeast cells.

With extract the range of stimulation is narrower, from 6.6×10^{-3} N to 4.0×10^{-3} N. In both cases the addition of normal solutions, or of greater concentrations, completely inhibit dehydrogenase catalyses.

Lanthanum nitrate

All concentrations of lanthanum from 2.0×10^{-1} N to 2.0×10^{-8} N increase the time of decolorization of methylene blue by yeast cells and by yeast extract. The inhibition is appreciable, however, only in concentrations of 2.0×10^{-2} N and greater.

Thorium nitrate

Dehydrogenase activity is reduced to zero by concentrations of thorium 0.2 N or greater. With a suspension of yeast cells this metal ion is stimulative in concentrations from 6.6×10^{-3} N to 2.0×10^{-3} N, or possibly 2.0×10^{-5} N (see table II). Greater amounts of the metal must be present in order to stimulate yeast extract, *i.e.*, concentrations from 4.0×10^{-2} to 6.6×10^{-3} N, but the stimulation, once induced, speeds up the reaction three or four times more than with yeast cells.

Discussion

Certain differences were regularly observed between the behavior of yeast suspensions and yeast extract. After the dye had been decolorized anaerobically with extract, the blue color could be restored only by prolonged and vigorous shaking in air. This may mean that the leucomethylene blue is adsorbed in some way which renders it inaccessible, possibly by occlusion to the maceration material. The autoxidation of leucomethylene blue to methylene blue proceeds less rapidly in control tubes of extract than in those containing the metals.

The decolorization by yeast extract is approximately three and one-half times more rapid than by yeast cells. Undoubtedly this means that when yeast cells are used, permeability is a factor of considerable importance. Grinding and extracting make the colloidal enzyme surfaces immediately available. However, when time of decolorization is plotted against the concentration of the metal added to the system, the resulting curves are similar whether yeast suspension or yeast extract has been used. This indicates that the effects of the ions on suspensions as well as on extracts is on the enzyme reactivity rather than on permeability.

Several experimental observations pointed to the association of inhibition with colloidal phenomena, especially adsorption. Metals manifest their inhibitive effects no matter when they are added to the dehydrogenase systems. Suppose a control tube, *i.e.*, one containing no metal, is decolorized. If, then, an inhibitive concentration of metal is added and decolorization allowed to proceed a second time, the formation of leucomethylene blue is

retarded. Presumably, metals inhibit dehydrogenase activity by diminishing the active catalyst surface.

The slight acceleration by traces of salts is not easily explained. It might be that small amounts of some of the added electrolytes exert an effect on the dispersion of the colloidal components of the cells and of the yeast extract. On the other hand, dilute solutions of some of the electrolytes might react selectively with the prosthetic group of the enzyme, whereas larger amounts exert a "blanket" effect on the enzyme carrier. This latter explanation, however, would not hold in all cases inasmuch as Ba, Mn, and K caused the greatest acceleration when present in high concentrations.

THUNBERG (**7, 8**) observed the effects of metallic salts on the rate of decolorization of indicators by oxidation-reduction systems of plant seeds. He found that low concentrations of Hg, Cu, and Ag accelerated, whereas high concentrations inhibited the decolorization of dyes by extracts of the seeds of *Trifolium pratense*. With other seeds on which Zn and Cd exerted a stimulative effect Ag had an indefinite action.

That a small fraction of a fatal concentration of certain metals may be used as a stimulant is an established physiological principle and has been observed by many investigators. Increasing concentrations of the metal become toxic. COPELAND (**1**) experimented with Elodea, Callitriche, a crucifer, a fish, and frog larvae, and found that small amounts of Cu, Zn, Cd, Ag, and Hg, stimulated respiration as measured by carbon dioxide evolution. VAN LAER (**10**) reached similar conclusions using yeast cells. LE VAN (**3**) found that low concentrations of Cu, Cd, Al, Na, Mg, and Fe increased respiration in *Lupinus albus*. The present investigation gives evidence that the stimulation which certain metals produce at low concentrations, and the inhibition which they produce at high concentrations, may be caused by a specific effect which these metals have on the dehydrogenase systems of the cells.

Summary

1. Using the methylene blue technique of THUNBERG the effects of KCl, KNO_3, $CuCl_2$, $MnCl_2$, $BaCl_2$, $AuCl_3$, $La(NO_3)_3$, $Th(NO_3)_4$ on the dehydrogenase of yeast and of macerated yeast extract were noted.

2. The order in which the cations inhibit the dehydrogenase activity of yeast cells is as folows:

$$Hg > Cu > Au > Th > La > Ba > Mn > K$$

3. The cations are inhibitive to the dehydrogenase system of yeast extract in the following order:

Hg > Cu > Au > Th > La > Ba. Mn and K show no inhibition.

4. At low concentrations four of the cations accelerate slightly when yeast cells are used.

$$Mn > Cu > Th > Au$$

5. With yeast extract Mn, Ba, and K accelerate dehydrogenase action markedly, especially in high concentrations. Th, Hg, Cu, and Au cause a slight acceleration in low concentrations, but inhibit strongly in high concentrations. Both high and low concentrations of Mn promote the catalytic activity of the extract. La in low concentrations has no effect, but inhibits in high concentrations.

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PECTIC CONTENT OF PLANT MATERIALS

WILLIAM E. ELWELL AND WILLIAM M. DEHN

Many plants and plant materials such as wood (**2**), tea (**25**), tobacco (**15**), cotton (**16**), hops (**13**), ramie (**11**), flax (**12**), grass (**6**), potato (**10**), plantain seed (**20**), celery (**9**), limes (**22**), guava (**1**), bergamot (**23**), and root hairs (**21**), have been shown to contain pectic compounds. Because different methods of extraction and analysis were employed, it is impossible to make comparative studies from these data of the pectic composition, types of pectic materials, and distribution of pectic compounds in these plants. A systematic study, employing uniform treatment of the pectic materials of leaves, cereal grains, apples, and citrus fruits, was conducted by NANJI and NORMAN (**18**). They determined water-soluble pectin, oxalic-acid-soluble pectin and ammonium-oxalate-soluble pectin. These data were interpreted as representing pectic material present as pectin, pectin in combination with metallic ions, and pectic acid. This interpretation was based on the theory that protopectin is an insoluble calcium, magnesium, or iron salt (**19**), and that treatment with oxalic acid and ammonium oxalate dissolved the pectin and pectates combined in this manner. More recent studies, however, (**3, 4, 14, 17, 24**), lead to the conclusion that the pectic molecule is essentially a long-chain, poly-galacturonic molecule combined through intermolecular forces and anhydride structure to form the insoluble protopectin layers of the cell wall and middle lamella. Single treatment with water or acid does not remove any definite pectic compound, but yields a part of a series of pectic substances; continued hydrolysis progressively dissolves the entire protopectin layer.

The lack of quantitative data for comparison of the distribution of pectin in the plant phyla has led to the present study. A large number of plants and plant materials have been included, and in order to obtain comparative data for the concentrations of each pectic fraction present, a system of extraction involving successive stages of hydrolysis was employed. The first stage involves the removal of the water soluble pectin; the second, the easily hydrolyzable protopectin; and the third, the difficultly hydrolyzable protopectin. These analyses have been supplemented by data on gelling power of the pectic material.

Extraction and analyses

One hundred grams of the material was ground in a sausage mill; 500 ml. of water were added, and the mixture was heated for one hour at 90° C., evaporated water being replaced during the heating. The pulp was then pressed free of the liquid and returned for a second extraction with 500 ml. of water containing 0.155 ml. H_2SO_4 (pH = 1.5). The pulp was again

TABLE I

PECTIN CONTENT OF VARIOUS PLANT TISSUES

PLANT	EXTRACT NO.	PECTIN AS CALCIUM PECTATE		PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	WET BASIS	DRY BASIS	
		%	%	%	%	
Apple (*Pyrus malus*)	1	0.37	2.46	0.22	1.36	Good
	2	1.00	6.67	0.21	1.35	
	3	0.23	1.37	0.18	1.20	
	Total	1.60	10.50	0.61	3.91	
Carrot (*Daucus carota*)	1	0.97	10.80	0.64	7.12	None
	2	0.33	3.68	0.62	6.89	
	3	0.21	2.34	0.41	4.56	
	Total	1.51	16.82	1.67	18.75	
Cranberry (*Vaccinium macrocarpon*)	1	0.31	2.21	0.38	2.71	Some
	2	0.34	1.64	0.22	1.57	
	3	0.04	2.85	0.12	0.86	
	Total	0.69	6.70	0.72	5.14	
Fern (*Pteridium aquilinum* var. *pubescens* Underwood)	1	0.57	1.78	0.17	0.53	Good
	2	0.37	1.16	0.76	2.37	
	3	0.21	0.61	0.26	0.81	
	Total	1.15	3.55	1.19	3.71	
Grape (*vitis vinifera*)	1	0.34	1.36	0.48	1.92	Some
	2	0.96	3.84	0.55	2.40	
	3	0.38	1.52	0.37	1.48	
	Total	1.68	6.72	1.40	5.80	
High bush cranberry (berry) (*Viburnum opulus*)	1	2.45	14.0	0.16	0.91	Good
	2	2.82	16.1	0.32	1.83	
	3	3.59	20.5	.....	.....	
	Total	8.86	50.6	0.48	2.74	
High bush cranberry (stored 3 months at 10° C.)	1	1.08	6.18	0.71	4.06	Slight
	2	1.28	7.32	0.62	3.54	
	3	0.53	3.03	0.38	2.17	
	Total	2.89	16.53	1.71	9.77	
Mountain ash (berry) (*Pyrus americana*)	1	0.90	2.57	0.22	0.63	Good
	2	0.96	2.74	0.98	2.79	
	3	0.88	2.51	.....	.....	
	Total	2.74	7.82	1.20	3.42	
Mountain ash (stored 3 months at 10° C.)	1	0.38	1.09	0.39	1.11	Slight
	2	0.19	0.54	0.49	1.40	
	3	0.01	0.03	0.21	0.60	
	Total	0.58	1.66	1.09	3.11	

TABLE I (*Continued*)

PECTIC CONTENT OF VARIOUS PLANT TISSUES

PLANT	EXTRACT NO.	PECTIN AS CALCIUM PECTATE		PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	WET BASIS	DRY BASIS	
		%	%	%	%	
Pea (hull) (*Pisum sativum*)	1	0.45	3.21	0.08	0.57	Good
	2	0.67	4.78	0.81	5.78	
	3	0.11	0.79	0.08	0.57	
	Total	1.23	8.78	0.97	6.92	
Scotch broom (*Cytisus scoparius*)	1	0.14	0.35	0.12	0.30	Good
	2	1.16	2.90	0.57	1.43	
	3	0.68	1.70	0.43	1.07	
	Total	1.98	4.95	1.12	2.80	
Snow berry (berry) (*Symphoricarpus racemosus*)	1	0.27	1.69	0.01	0.06	Some
	2	0.53	3.31	0.41	1.60	
	3	1.50	9.37	1.00	6.25	
	Total	2.30	14.37	1.42	7.91	
Sow thistle (*Sonchus oleraceus*)	1	0.37	2.18	0.07	0.41	Good
	2	0.72	4.24	0.42	2.47	
	3	0.35	2.06	0.18	1.06	
	Total	1.44	8.48	0.67	3.94	
St. John's wort (bud) (*Hypericum calycinum*)	1	1.10	4.08	0.13	4.82	Good
	2	2.69	9.62	5.03	18.60	
	3	1.17	4.34	0.69	2.56	
	Total	4.96	18.04	5.85	25.98	
St. John's wort (stem)	1	0.83	1.93	0.06	0.14	Good
	2	1.18	2.75	2.24	7.27	
	3	0.92	2.14	0.54	1.25	
	Total	2.93	6.82	2.84	8.66	

pressed and returned for a third extraction; the same quantity of the acid solution previously used was added and a 2-hr. extraction was employed. The filtrates obtained from the three extractions were each made up to one liter, and aliquot parts were used for analysis. Data on the pectin content of various plants, as determined both by the method of calcium pectate precipitation (**22**) and by the method of alcohol precipitation, are given in table I, with calculations to the dry basis. Data on the pectin content of vegetables, obtained by the method of alcohol precipitation, are shown in table II with calculations to the dry basis. Data on the pectin content of apples according to this method of treatment are included in table I to serve for comparison with other published analyses.

TABLE II

PECTIC CONTENT OF VARIOUS VEGETABLES

PLANT	EXTRACT NO.	PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	
		%	%	
Artichoke (*Cynara scolymus*)	1	1.28	7.12	Slight
	2	0.87	4.83	
	3	0.34	1.89	
	Total	2.49	13.84	
Asparagus (*Asparagus officinalis*)	1	0.29	4.15	None
	2	1.56	22.10	
	3	0.16	2.29	
	Total	2.01	28.54	
Beet (top) (*Beta vulgaris*)	1	0.32	6.40	None
	2	0.43	8.63	
	3	0.41	8.20	
	Total	1.16	23.23	
Beet (root)	1	0.32	2.00	None
	2	0.47	2.94	
	3	0.39	2.44	
	Total	1.18	7.38	
Broccoli (*Brassica oleracea*)	1	0.84	6.46	None
	2	1.07	8.15	
	3	0.54	4.16	
	Total	2.45	18.77	
Brussels sprout (*Brassica oleracea* L. var. *gemmifera* Zenker)	1	0.69	3.83	None
	2	0.94	5.22	
	3	0.81	4.50	
	Total	2.44	13.55	
Celery (*Apium graveolens*)	1	0.40	5.72	None
	2	0.44	6.28	
	3	0.15	2.14	
	Total	0.99	14.14	
Dandelion (*Taraxacum officinalis*)	1	2.11	8.80	None
	2	2.21	9.22	
	3	3.15	13.10	
	Total	7.47	31.42	
Lettuce (*Lactuca sativa*)	1	0.13	4.33	Slight
	2	0.28	9.34	
	3	0.24	11.32	
	Total	0.75	24.99	
Onion (*Allium cepa*)	1	0.36	3.00	None
	2	0.72	6.00	
	3	0.32	2.67	
	Total	1.40	11.67	

TABLE II (*Continued*)

PECTIC CONTENT OF VARIOUS VEGETABLES

PLANT	EXTRACT NO.	PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	
		%	%	
Parsnip (*Pastinaca sativa*)	1	0.83	5.22	None
	2	1.10	6.88	
	3	1.20	7.50	
	Total	3.13	19.60	
Potato (*Solanum tuberosum*)	1	0.81	3.52	None
	2	1.17	5.08	
	3	0.77	3.35	
	Total	2.75	11.95	
Rhubarb (*Rheum rhaponticum*)	1	2.42	30.4	Slight
	2	2.84	35.5	
	3	2.00	25.0	
	Total	7.26	90.9	
Rutabaga (*Brassica napobrassica* Mill.)	1	0.39	2.17	None
	2	0.71	3.97	
	3	0.47	2.61	
	Total	1.57	8.75	
Spinach (*Spinacea oleracea* L.)	1	0.96	3.00	None
	2	0.47	3.91	
	3	0.61	5.08	
	Total	2.04	11.99	
Squash (*Cucurbita moschata*)	1	0.20	1.82	None
	2	0.50	4.54	
	3	0.80	7.28	
	Total	1.50	13.64	
Sweet potato (*Ipomoea batatas*)	1	1.63	4.80	None
	2	0.99	2.91	
	3	0.95	2.79	
	Total	3.57	10.50	
Swiss chard (*Beta vulgaris* var. *cicla* L.)	1	0.22	4.40	None
	2	3.17	63.40	
	3	0.15	3.00	
	Total	3.54	70.80	
Tomato (*Lycopersicum esculentum*)	1	0.32	5.33	None
	2	0.19	3.17	
	3	0.11	1.84	
	Total	0.62	10.34	
Turnip (*Brassica campestris* var. turnip)	1	0.44	3.38	None
	2	0.39	3.00	
	3	0.44	3.38	
	Total	1.27	9.76	

Tests of the gelling power of the alcohol precipitate were made as follows: The filtrate from an one-hour extraction of 100 gm. of the material with 500 ml. of water containing 0.155 ml. H_2SO_4 was added to two volumes of 95 per cent. alcohol. The precipitated pectin was separated, dried, and one gram of it with one gram tartaric acid was dissolved in 55 ml. of water, and 100 gm. of sucrose added. The mixture was boiled until a weight of 150 gm. was attained. The strength of the jelly was estimated after standing for 24 hours.

Discussion

The data of tables I and II disclose several interesting points concerning concentration, properties, and changes of pectin in plant materials.

Among plants of the same family or genus there is no apparent correlation in amount of the three types of pectic material. In the case of *Pyrus malus* the predominant type is that obtained by hot water extraction, while *Pyrus americana* shows approximately equal concentrations of each type. Certain correlation is exhibited by the members of the genus Brassica in the predominance of the hot water soluble pectin.

All the plants studied are rapidly growing and BUSTON'S (**5**) hypothesis that pectic materials develop under conditions of rapid growth is confirmed. The table shows that slower growing, woody plants, such as Scotch broom, sow thistle, and bracken fern contain but small quantities of pectic material while the fast growing vegetables contain large quantities. On the other hand, it is evident that conditions of high pectin content and fast growth do not favor the formation of the gelling type of pectin. Plants that possess pectin of high gelling power usually contain less than 10 per cent. of pectin on the dry basis, while those of low gelling power are of the vegetable type and contain more than 10 per cent. pectin.

The plant pericarps, such as those of fruits and berries, are the usual source of gelling pectin. It is shown that stems and branches of plants may also yield pectin of the gelling type, as in the cases of Scotch broom, bracken fern, sow thistle, and St. John's wort. Other sources of gelling pectin are mountain ash, high bush cranberry, cranberry, snow berry, St. John's wort buds, and pea-hulls.

The natural process of pectin alteration in plants follows a course of protopectin hydrolysis followed by hydrolysis of soluble pectic compounds and pectates (**7, 8**). From data concerning the change of pectic material during storage in mountain ash, and high bush cranberry, it is concluded that during ripening the type of pectin that is obtained by the third extraction undergoes the most rapid change of the three types of pectic compounds. There is an accompanying decrease in amount of the pectic material of the types derived by the first and second extraction processes. The rate of decrease in these types is less, indicating that the enzymatic processes in-

volved in the degradation of the difficultly soluble type of pectic material proceed more rapidly than those that effect the soluble type. This may be attributed to different enzymatic actions, or, to a single process, the rate of which changes with altering conditions.

Summary

Comparative quantitative data on the pectic content of many plant materials have been obtained; also, data indicating the enzyme processes involved in the degradation of pectin.

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PRODUCTION AND CONSUMPTION OF ETHYLENE BY ETHYLENE-TREATED BANANAS

R. C. Nelson

(WITH TWO FIGURES)

Introduction

In an earlier paper (**2**) the author has described the emission of ethylene by bananas into a closed chamber during the ripening process. The effect of added ethylene on this process is herein described.

Materials and methods

Bananas were ripened under the same conditions as in the earlier experiments. They were taken off the car as they arrived green in Minneapolis, and the individual "fingers" were separated from the hands and washed. 4.46 kg. of fruit were arranged on a wire stand over which was placed a bell-jar with a capacity of 14.8 liters. Within the bell-jar was a vessel holding 100 ml. of 7.5 N sodium hydroxide solution to absorb any carbon dioxide produced. The carbon dioxide absorbed was replaced by oxygen under low constant pressure from a gasometer, thus maintaining approximately normal atmospheric conditions under the bell-jar while permitting the collection of emanations. The whole was kept in a room where the temperature varied from 18° C. to 19° C. At the end of each day the fruits were uncovered and allowed to air for about an hour.

At the beginning of each day's run, immediately after the bananas had been enclosed in the bell-jar, cylinder ethylene of anaesthetic grade was measured into the jar in quantities ranging from 1.15 to 1.25 ml., using a micro gas burette. At the end of two hours, which time was found ample to permit diffusion of ethylene throughout the bell-jar, a 330-ml. sample of gas was removed from the bell-jar and its ethylene content determined. This value was taken as the initial concentration of ethylene in the atmosphere surrounding the bananas. The final concentration was determined the next day on a similar gas sample taken just before the bell-jar was opened to air the bananas.

Ethylene determinations were made according to the author's method (**2**). The carbon dioxide production of the bananas was measured by diluting the sodium hydroxide which had been placed in the bell-jar to 1000 ml., and titrating a 25-ml. aliquot with 0.5 N hydrochloric acid after the addition of sufficient barium chloride solution to precipitate the carbonate.

The volume of the bananas was used in certain calculations. This value, calculated from the specific gravity determined by the conventional water-displacement method on whole fruits, was found to be approximately 0.945.

The bell-jar was tested for its ability to retain ethylene. To it was added 1.35 ml. of ethylene. A sample was withdrawn after two hours; and when an ethylene determination was made on it, it was found to titrate 0.81 ml. of 0.002 N potassium permanganate solution. Twenty hours later the titre of a sample was 0.80 ml. The titre required by calculation from the first day's determination was 0.79 ml. Evidently losses of ethylene from the bell-jar may be disregarded.

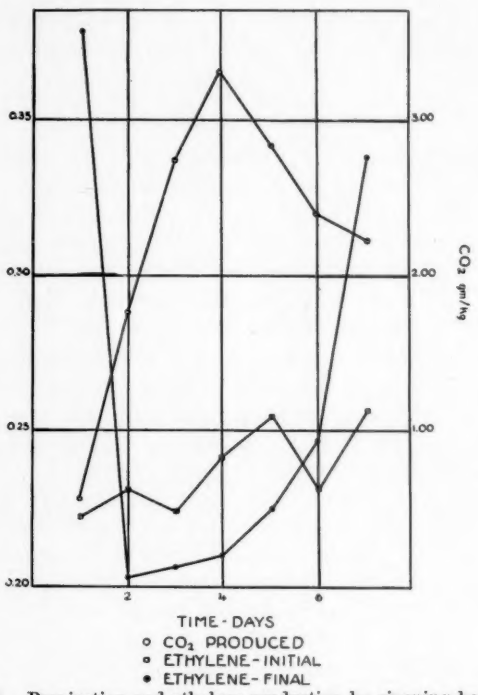

FIG. 1. Respiration and ethylene production by ripening bananas.

Results

The results obtained are shown graphically in figure 1. It will be observed that the bananas were received just as they entered the climacteric period. At this time they were grass-green, greener than bananas are usually received at Minneapolis. By the end of the fourth day the green color had almost entirely disappeared. At the end of the fifth day the characteristic full aroma of ripe bananas was present, although the fruits were still quite firm, and even on the sixth day they had not reached the softness characteristic of fully ripe bananas.

The respiration increased rapidly to a maximum on the fourth day, after which it declined at a somewhat slower rate.

On the first day the bananas emitted about 0.16 mg. of ethylene per kg. into the air of the bell-jar where the initial concentration of ethylene was 73 p.p.m., which indicated a high concentration of ethylene in the interior of the fruit. On the second day and on the three days thereafter the movement of ethylene was in the opposite direction. On the sixth and seventh days ethylene was again emitted into the air of the bell-jar.

In table I is presented the analytical data for the initial concentrations of ethylene in the bell-jar together with the doses of ethylene applied.

TABLE I

Day	Volume of air	Titration $KMnO_4$	C_2H_4 determined	C_2H_4 added	Percentage recovery
	l.	*ml.*	*mg.*	*mg.*	%
1	10.1	0.99	0.99	1.47	67.0
2	10.1	1.03	1.03	1.51	68.1
3	10.1	1.00	1.00	1.47	67.7
4	10.5	0.94	0.975	1.42	68.5
5	10.5	0.99	1.03	1.49	69.0
6	10.5	0.90	0.935	1.45	64.8
7	10.6	0.96	1.005	1.51	66.8
8	14.8	0.81	1.19	1.69	71.0

The value "mg. ethylene added" was calculated from a volume which had not been corrected for temperature and pressure, and the actual percentages of recovery would be about 10 per cent. higher. The reason for the low recovery shown in the last column of table I is not certain, but it is prob-

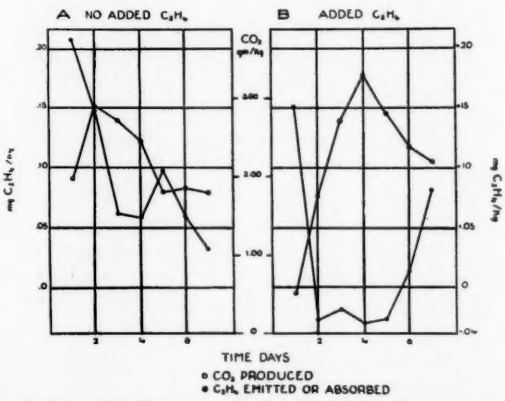

FIG. 2. Respiration and ethylene exchange of ripening bananas in the presence and absence of added ethylene.

ably caused by adsorption of ethylene on the walls of the bell-jar. The relatively large surface of the jar and the small quantity of ethylene added make this explanation plausible. When ten times the quantity of ethylene is added, and a smaller sample taken the recovery is almost quantitative. Evidently about as much ethylene is lost during airing as is adsorbed during the two hours which elapse between giving the dose of ethylene and withdrawing a sample for determination. The overnight leakage trial showed that no ethylene was lost from the atmosphere after the first two hours.

The adsorption of the odorous constituents of banana on the bell-jar is easily demonstrated. If after airing until no further aroma of banana can be detected in the jar, it is closed again, apart from the bananas, after a short time the banana odor may be detected in it.

Discussion

Figure 2 shows a comparison of runs made on bananas under similar conditions with and without added ethylene. In an earlier paper (**2**) the author suggested that the rise in ethylene emission on the fifth day (fig. 2A), coincident with the end of the ripening process, might be an indication that ethylene is consumed in the ripening process in banana. The results obtained in the present experiment seem to support this view.

A more complete demonstration of the consumption of ethylene might have been obtained by determining the residual ethylene present in the bananas. The extent of loss of ethylene entailed in the sampling process, however, is not known and it was felt that the significance of such analyses might not be great. Furthermore, this procedure would cause a considerable daily decrease in the banana tissue beneath the bell-jar, which is undesirable.

Some ethylene is lost from the fruits during the 1-hour airing period. A comparison, however, of the percentage recovery of ethylene in control determinations with that in determinations with bananas in the bell-jar indicates that only about 3 per cent. of the ethylene dose penetrates into the bananas in the 2-hour period allowed for diffusion. It seems likely that the loss during airing is of about the same magnitude. Even if all the ethylene in the bananas were lost, however (about 10 per cent. of the dose), the analyses would still show complete inhibition of ethylene emission during active ripening. Only disappearance of ethylene within the fruit could account for this, since the ethylene emitted by untreated fruits is far more than sufficient to raise the internal concentration to a level equal to that of the external treatment.

It must therefore follow that, since the ethylene does not escape from the bell-jar, it must either be consumed by the fruit or bound by it in some manner. There are strong objections to the latter hypothesis. During the

first day, much ethylene diffused out into the air space; therefore, any adsorbent for ethylene present in unripe fruit must have become saturated during the first day. Following the same line of reasoning, one is eventually driven to the conclusion that only some transient product of the ripening process could be involved, and the rise in ethylene emission when the ripening process becomes complete is hardly sufficient to account for all of the ethylene which must have been bound during ripening. Ethylene therefore must disappear by chemical change during ripening.

Although both respiratory and hydrolytic processes go on at a high rate during ripening, there is reason to believe that ethylene is consumed in the latter processes rather than in the former. In neither graph (fig. 2) is there any evidence of a direct relationship between ethylene consumption and respiratory activity. It is true in a general way that ethylene consumption is great when respiration is high, but widely different respiratory rates may correspond to the same degree of ethylene consumption.

WOLFE (5) has investigated the rate of hydrolytic processes during banana ripening. He finds that the rate of hydrolysis of starch plotted against time gives a somewhat symmetrical curve with a maximum at about the same time as the respiratory maximum. Evidently such a curve resembles much more closely the ethylene consumption curve.

Even more significant is the observation of WOLFE that while ethylene treatment caused little change in the total respiratory activity over the ripening period, it did produce consistent increases in the rate of starch hydrolysis.

All the evidence at hand points, therefore, to a connection of ethylene with the hydrolytic rather than the respiratory processes in ripening bananas, although it may act also as a stimulator of the latter.

Nothing is known at present which suggests a mechanism for the stimulation of starch hydrolysis by ethylene, or its consumption therein. REA and MULLINIX (3) state that ethylene is able of itself to act as a catalyst for the hydrolysis of starch. The author has repeated this work, with a modification. REA and MULLINIX measured the extent of hydrolysis of a starch suspension which had been saturated with ethylene, by a method involving the reduction of Fehling's solution. It was considered that hot alkali was a rather drastic treatment to which to subject starch, and the hypoiodite method of WILLSTÄTTER and SCHUDEL (4) was selected to determine the amount of hydrolysis, on the basis that the possibility of side reactions was less. Under these conditions no hydrolysis of corn starch with or without ethylene was detectable at the end of a week. If a comparison of the two experiments is justifiable, it might be concluded that in the work of REA and MULLINIX ethylene had rendered the starch more susceptible to alkaline hydrolysis. In any case it may be said that ethylene alone cannot cause

the hydrolysis of starch. Nor does ethylene have any influence on the saccharifying activity of taka-diastase according to ENGLIS and ZANNIS (1).

The results of the present experiment explain the acceleration by ethylene of the ripening of fruits which are already producing considerable amounts of ethylene, and confirm the previously offered explanation for the shape of the ethylene emission curve for ripening bananas.

Conclusions

Ethylene is consumed by the bananas in the course of the ripening process, probably in connection with the hydrolysis of starch. Ethylene treatment of fruits already producing ethylene is believed to accelerate ripening because of this fact.

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EVIDENCE FOR THE ESSENTIALITY OF SILICON FOR GROWTH OF THE BEET PLANT[1]

G. J. RALEIGH

(WITH ONE FIGURE)

Introduction

In experiments as yet unpublished, difficulty was encountered in growing plants of the Detroit Dark Red variety of the table beet (*Beta vulgaris* L.) in culture solutions of low concentration in asphalt-painted iron containers. When the plants were transferred from solutions made with a distilled water contaminated with tap water to solutions made with uncontaminated distilled water, the plants in lower concentrations of nutrient salts wilted badly within a few days after being transferred, and the roots became dark in color. Those in higher concentrations made satisfactory growth, which suggested the possibility that some necessary element was being supplied as an impurity in the C.P.-grade chemicals. Preliminary experiments with varying quantities of the four chemicals employed in making the solutions indicated that the impurities might be contained primarily in the ammonium phosphate.

Since A5 (B, Cu, Mn, Zn, Mo) and B6 (Ti, V, Cr, W, Co, Ni) chemicals[2] had been supplied, it was thought possible that the missing nutrient might be one contained in the C13 mixture used by ARNNON (1) (Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be). Single plants from the concentrated solutions were transferred to Mason jars coated with a thin layer of asphalt paint and containing nutrient solutions one-half as concentrated as those used by ARNON. The plants were supplied with various combinations of the 13 micro-elements. Others received small quantities of sodium, chlorine, and increased quantities of B, Cu, Mn, Zn, V, Cr, W, Co, and Ni.

The solutions were not aerated. After a few days the plants wilted irrespective of treatment. The possibility existed that beet plants might have a high requirement for oxygen in the solution and for that reason these plants were kept for comparison with those grown in metal containers in aerated solutions. After 3 weeks the roots of the plants in the Mason jars were making good growth and were lighter in color than any of those in the metal containers. This observation suggested that some nutrient was dif-

[1] The writer is especially grateful to Professor D. R. HOAGLAND for making the facilities of the Division of Plant Nutrition available for the work and for his interest and suggestions, to Mr. P. R. STOUT for suggesting the method and preparing the K_2SiO_3 used in experiment 4, and to Drs. MAX GARDNER and C. M. TOMPKINS for suggestions concerning the control of damping-off of the seedlings in sand. This work was done while on sabbatic leave from Cornell University.

[2] For the composition of similar A and B supplementary solutions see citation (1).

fusing from the soft glass through the asphalt coating. Trial experiments were set up using 10 p.p.m. of Si as K_2SiO_3 and aluminum as aluminum sulphate at the rate of 1 p.p.m. and of 0.5 p.p.m.—much in excess of the amount of that element supplied in the C13 chemicals. After several days all of the plants except those supplied with silicon wilted as previously described. Other experiments were conducted in unpainted Pyrex beakers. Plants not supplied with Si were markedly checked in growth during the first 10 days. Later the difference in growth rate between these plants and those receiving Si disappeared, probably owing to Si dissolved from the glass by the solution. These experiments indicated that slow growth without Si was not associated with the asphalt paint used on the iron containers.

On the basis of these suggestions a series of experiments was carried out to study the relation of Si to the growth of the beet plant. In order to avoid the added impurities in more concentrated solutions the plants were grown in dilute solutions of the following composition: $NH_4H_2PO_4$, 0.0005 M; $MgSO_4$, 0.001 M; $Ca(NO_3)_2$, 0.002 M; KNO_3, 0.003 M. There is a large amount of evidence (**2**) that such solutions give as good results as more concentrated solutions if the plants are not grown for long periods without changing the solutions.

In all cases the solutions were made from the dry, C.P.-grade salts immediately before use. A and B mixtures of micro-elements were added to supply elements which are known to be required as well as others which may possibly have a function in plant nutrition. When silicon was supplied its source, except in experiment 4, was pure grade K_2SiO_3. Iron was added three times weekly. In all experiments an attempt was made to maintain the pH of the solutions between 6.1 and 6.5.

Seed was treated with 3 per cent. formalin for 10 minutes and planted in coarse sand. Except where indicated the experiments were carried out in asphalt-painted 15-liter iron containers supplied with pin-punctured rubber tube aerators and asphalt-painted plaster of Paris tops.

Procedure

Experiments 1 and 2

Plants grown in solutions with silicon before experimentation.—Seed was sown December 22, and 10 seedlings for each container were transferred to solutions containing 10 p.p.m. Si on January 9. On January 17, when the plants averaged 3.6 cm. in height, they were changed to solutions without silicon and solutions which had a concentration of 17 p.p.m Si. Data given in table I were taken February 13.

Other plants of the same lot were grown in solutions containing 10 p.p.m. Si until February 17 when they were 17 cm. in height before being transferred to solutions with 15 p.p.m. Si and to others without Si. Six plants

were set out in each container. They were harvested March 11. Results are given in table I.

TABLE I

EFFECT OF ADDING SILICON ON THE GROWTH OF BEETS IN CULTURE SOLUTIONS. AVERAGE FRESH WEIGHT IN GRAMS

TREATMENT	TOPS	BEETS	ROOTS CENTRIFUGED
	gm.	*gm.*	*gm.*
Experiment 1			
with Si 1	3.15	.....	0.85
with Si 2	3.24	.....	0.80
without Si 1	0.99	.....	0.46
without Si 2	0.63	.....	0.34
Experiment 2			
with Si 1	32.78	9.05	5.92
with Si 2	35.02	12.87	6.36
without Si 1	11.22	3.68	3.31
without Si 2	7.70	3.12	2.90
Experiment 3			
with Si 1	2.51	.....	0.51
with Si 2	2.20	.....	0.48
without Si 1	0.26	.....	0.11
without Si 2	0.28	.....	0.10
Experiment 4			
with Si 1	1.55	.....	0.28
with Si 2	1.15	.....	0.21
without Si 1	0.36	.....	0.10
without Si 2	0.25	.....	0.06

EXPERIMENT 3

YOUNG PLANTS.—Seed was sown January 7, and 22 plants to the container were transferred to experimental solutions on January 20, as soon as they had reached sufficient size to make the transfer practicable. At that time the cotyledons had a spread of 2 cm. A total of 19 p.p.m. Si in the nutrient solution was supplied to those plants which received silicon. The plants were harvested February 20 with results as shown in table I (see also fig. 1).

EXPERIMENT 4

PURIFIED SILICON.—Since the K_2SiO_3 used in experiments 1, 2, and 3 might have contained impurities, the work was repeated using K_2SiO_3 prepared by distilling off SiF_4 produced by the reaction of HF on amorphous SiO_2, hydrolyzing the SiF_4 in water, neutralizing with NH_4OH, and filtering off a large part of the F as NH_4F. The remaining F was removed by heating to 850° C. The residual SiO_2 was weighed and converted to K_2SiO_3 by heating with the equivalent amount of K_2CO_3. The SiF_4 was collected

in platinum ware. All parts of the procedure beyond that point were carried on in platinum.

Plants of the same lot as those used in experiment 3 were transferred on January 24 to solutions containing 10 p.p.m. Si and on February 4 when they averaged 4 cm. in height were again shifted, some to solutions without Si and others to solutions with 8.3 p.p.m. Si furnished by the purified K_2SiO_3. The plants were harvested February 18. Although the period of the experiment was short, the differences in the weights of plants grown with silicon and those without were marked as shown by the data in table I.

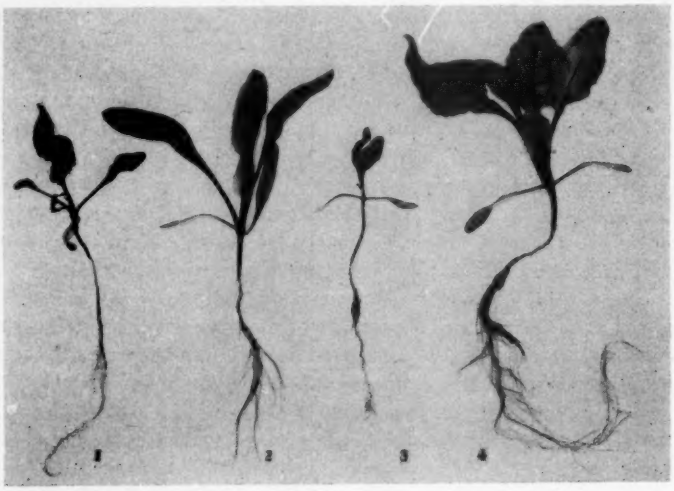

FIG. 1. Plants grown with and without Si in culture solutions. One and 3 without Si, 2 and 4 with Si; 1 and 2 from experiment 4, 3 and 4 from experiment 3.

SYMPTOMS OF SILICON DEFICIENCY IN THE BEET

The response to the silicon used in experiments 1, 2, and 3 and to that supplied in experiment 4 was apparently the same. Plants made rapid growth with green foliage (including the cotyledons), and straw colored roots. Plants in solutions deficient in silicon made very slow growth. After about 3 days in such solutions the roots became slightly darker in color and after a considerable time very dark. At this stage the roots were often covered with a growth of organisms, probably owing to the food supplied by the dying roots. On older beets secondary growth of roots was common. When plants were transferred to silicon-deficient solutions at an early stage, damping-off was common after a considerable time in the solutions. When silicon was added, practically no damping-off occurred.

As indicated previously, wilting of the outer leaves during periods of high transpiration was one of the early symptoms of silicon deficiency. These leaves developed anthocyanin along the veins including the secondary veins. In younger plants the cotyledons turned yellow and in most cases soon died.

Discussion

A large amount of work has been done regarding the essential nature of silicon and its possible relation to phosphorus in the nutrition of plants. SOMMER (**5**) improved the growth of rice and of millet by the addition of silicon to the culture solution. LIPMAN (**4**) found that sunflower and barley were definitely benefited, especially as regards seed production by the presence of silicon in the culture medium. ISHIBASHI (**3**) reported increased growth of rice grown in pots when air-dried silicic acid was added to the soil. In a previous experiment he obtained increased yield of rice in water culture when Si was added to the nutrient solution.

The present investigation indicates that silicon plays an important rôle in the growth of the table beet in culture solutions, and in fact it seems to be indispensable. That the response to silicon in these experiments was more marked than that reported by earlier investigators may be explained on the assumption that the beet has a higher requirement for Si than plants previously studied. Possibly also the chemicals used in some of the earlier experiments may have contained considerable silicon as an impurity. In other cases the coatings of the glass containers may have allowed silicon to diffuse from the containers into the solutions.

Under some conditions phosphoric acid reacts with silicon compounds. In this work the ammonium phosphate carried a major part of the silicon impurities. Conceivably in some of the experiments in which silicon was beneficial when used with very low quantities of phosphorus in the nutrient solution and the benefit attributed to the influence of Si on the utilization of phosphate by the plant, the response was in reality a direct response to silicon.

Summary

Experiments are reported which indicate that silicon is an indispensable chemical element for growth of the beet plant.

CORNELL UNIVERSITY
ITHACA, NEW YORK

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BRIEF PAPERS

A METERING PUMP FOR SMALL VOLUMES OF SOLUTION

M. A. RAINES

(WITH ONE FIGURE)

The method usually employed in botanical laboratories for supplying a regulated flow of solution to a culture vessel is by the use of a capillary resistance device, as summarized by ZINZADZÉ (**3**). Another method, recently employed by the writer (**1**), is by the use of wicks disposed over the edge of a constant level tank. Both methods are extremely simple. Many workers, however, find them quite onerous on account of the developed skill and dexterity required for adjusting the rate of the solution flow, and the liability to decrease or stoppage of flow owing to the clogging of the small orifices or fine capillary channels employed. For such workers the use of a metering pump is indicated.

The metering pump here described was devised for use in connection with the modified set-up for wick culture (**2**). It is believed that as described, or in modified form, it will probably be found advantageous also in other situations in the laboratory. It will be noted that the pump has only one moving part. There are no valves nor tripping mechanism which may get stuck, and no small orifices which may become clogged. The solution, in its path through the apparatus, need not come into contact with any material other than glass.

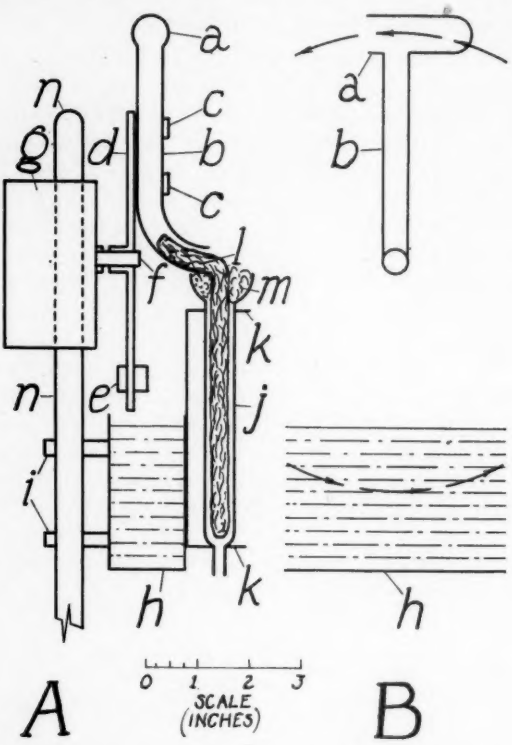
The essential features of construction of the apparatus are illustrated diagrammatically in the accompanying figure, in which A is a view from the side, showing details of the entire assembly, and B a representation as seen from the front of only the measuring cup (a) with its outflow tube (b) and the constant level tank (h). The arrows in B indicate the path of travel of the measuring cup—through a circular path in a vertical plane. At the bottom of this path the cup is dipped into the solution contained in the constant level tank (h), and towards the top of the path the liquid contained in the cup drains down the outflow tube (b) and out its open end into the funnel tube (j), from which it flows into the culture vessel below (not shown).

The pump is driven by a small synchronous electric motor (g)[1] mounted by means of clamps (not shown) on the rod (n) of a laboratory stand. This motor is sufficiently powerful to handle measuring cups of a capacity up to 10 ml. The capacity of the cup may be reduced any desired amount by the simple expedient of inserting wads of glass wool into it. Regulation of rate

[1] A "time machine" obtained from the Hansen Manufacturing Co., of Princeton, Indiana. These small motors cost less than $2.00 each, and are obtainable from stock in a variety of set speeds from 4½ revolutions per minute to 1 revolution per day.

of flow of solution is also accomplished by substituting motors of different speed.

As the open end of the outflow tube is directed horizontally, the solution often does not drain out of it completely. This difficulty may be obviated by introducing a wick (l)[2] of spun glass into the open end of the outflow tube,



the lower end of the wick being suspended in the funnel tube (j). Wads of glass wool (m) at the upper end of the funnel tube are used to hold the wick in place. Such a glass wick operates excellently to effect complete drainage of solution from the outflow tube. Its use facilitates compactness of construction of the apparatus, and precision of delivery of the measured quantity of solution (especially important when a measuring cup of small capacity, as 0.5 or 1 ml., is being used).

The measuring cup (a) with its outflow tube (b) is attached by means of

[2] Made from strands of glass rope obtained from the Plymouth Cordage Co., North Plymouth, Mass.

spring clips (fuse clips) to the carrier arm (d) which is mounted on the motor shaft (f). In mounting the measuring cup on the carrier arm, it is desirable that the open end of the outflow tube be well centered, in line with the motor shaft. At the other end of the carrier arm is an adjustable counterweight (e). The funnel tube (j) rests on brackets (k) attached to the wall of the constant level tank (h). This in turn is mounted on the rod (n) of the laboratory stand by means of the clamps (i).

Summary

A bucket type pump is described for delivering small measured volumes of solution. It has only one moving part, and no small orifices. A glass wick may be used for draining the solution from the open end of the outflow tube. The solution, in its path through the pump, need not come into contact with any material other than glass.

HOWARD UNIVERSITY
WASHINGTON, D. C.

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METERING PUMP AND TURNTABLE ARRANGEMENT FOR SUPPLYING MEASURED QUANTITIES OF NUTRIENT SOLUTION SUCCESSIVELY TO ANY NUMBER OF CULTURE VESSELS[1]

H. G. DU BUY

(WITH FIVE FIGURES)

In connection with experiments in plant nutrition, especially the plant physiological worker frequently has the task of supplying definite quantities of solution, in a given time, to each of a number of culture vessels. The apparatus here described was constructed to effect this in the case of an experiment in which the plants are being grown in sand in stoneware jars. Each experimental set consists of 12 jars, intended to receive equal quantities of the same nutrient solution. The 12 jars of a set are placed on a wooden bench.

The new arrangement replaces a capillary drip method previously employed. The change in method has effected a considerable saving in time required for adjusting and servicing the supplying of nutrient solution to the culture jars. It is recognized that there are individual differences of facility and dexterity in laboratory techniques, and also that preferences in, and successes with, laboratory procedures are not without a subjective element.

FIG. 1. Photograph of the apparatus in use.

[1] Contribution no. 496, Department of Botany, the University of Maryland.

Solution measuring mechanism

This is an adaptation to the present situation of the metering pump for small volumes of solution devised by RAINES[2] of Howard University in connection with the modified set-up for wick culture developed by him and described in the preceding paper. Figure 1 is a photograph of the entire mechanism, and figure 2 a front view. The motor (m, fig. 3) turns a cup (a),

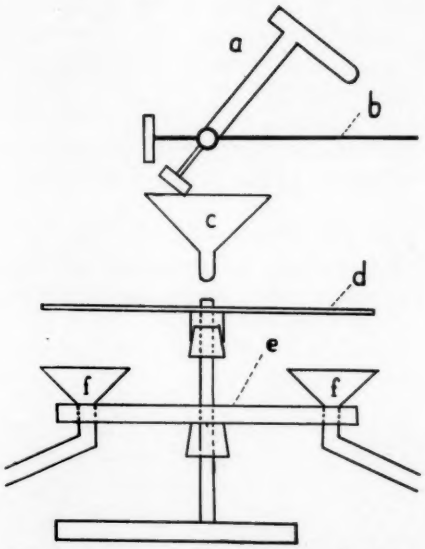

FIG. 2. Front view of the whole mechanism.

which, due to the gradual slope of the outlet tube, deposits the water in a receiver funnel (c). The gradual slope of the cup tube foregoes the use of a wick in order to drain the tube, and also the precise centering of the outlet.

Figure 3 shows a side view. Container (v) is connected by glass tubing with a reservoir (not shown in fig. 3) which serves as a constant level device. For instance, a turned-over 5-gallon carboy with trough can be placed at any suitable point and the liquid from there administered to the container. The water of the cup drops into a receiver.

This modified metering pump has been used in connection with a turntable and a funnel system instead of a receiver with a wick system which was unsatisfactory in the use of nutrient solutions.

[2] Raines, M. A. Plant Physiol. 14: 829–831. 1939.

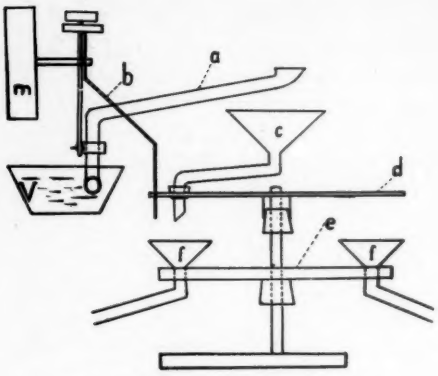

FIG. 3. Side view of the mechanism.

The distributor mechanism

The receiver funnel (c) (fig. 4) is attached to a turntable (d). This turntable, which turns on a metal bearing, has as many dents as there are

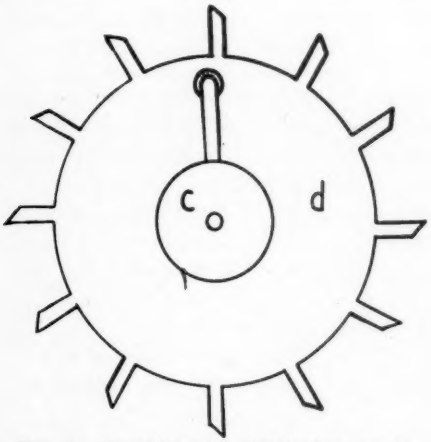

FIG. 4. Turntable with distributing funnel.

cultures to supply. Each dent catches the turn axis (b) which is turned by the same motor that turns cup (a). By this means the outlet of receiver funnel (c) is moved serially on top of each small funnel (f^1, f^2, f^3, etc.) of funnel table (e) (fig. 5) which is stationary. The funnels supply the liquid to the cultures. They can be easily removed from the slits (g^1, g^2, g^3, etc.) of funnel table (e).

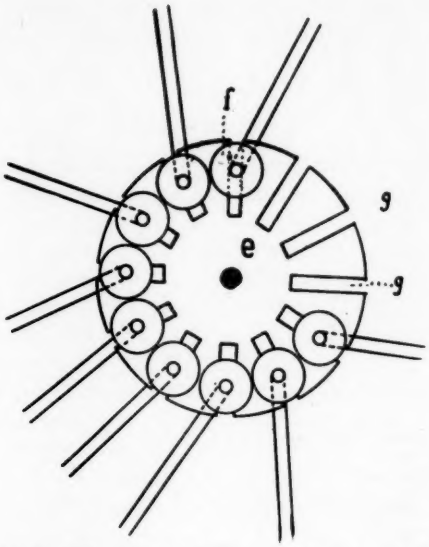

FIG. 5. Funnel table with notches and some secondary funnels going to the jars.

The operation of the apparatus is made more clear in the figures. Except for the motor all parts are made of easily obtainable material: the turntable (fig. 4), as well as the turning axis, is made of galvanized iron. Standard ring stands are used. In order to vary the height of the distributor, a secondary ring-stand rod may be used for a carrier and may be made adjustable by attaching this rod to the ring stand with a clamp. The metal bearing may be made of the brass part of a fuse and attached to a cork. The funnel table may be made of wood or Celotex. The glass parts can be painted in order to prevent development of algae.

The advantages of the apparatus are:

1. The water or culture solution comes into contact only with glass.
2. The amount of liquid to each jar is well defined and can be varied at will.
3. The wide tubing used in the whole system prevents all clogging even after long periods.
4. All glass parts may be easily removed for cleaning and are easily adjustable.
5. Since only the constant level reservoir has to be filled after long intervals, the method is not time consuming.

UNIVERSITY OF MARYLAND
COLLEGE PARK, MARYLAND

CONVENIENT SEEDLING SUPPORT FOR GROWING PLANTS IN WATER CULTURE[1]

STUART DUNN

(WITH ONE FIGURE)

Difficulties are frequently encountered with the usual method of starting seedlings in water culture for nutrient solution studies. This is conventionally done by placing the sterilized and soaked seeds on a paraffin-impregnated mosquito netting stretched tightly over the top of a jar filled with either water or dilute nutrient solution. After the seedlings have reached a convenient size (about 2 or 3 inches high, if wheat is being used), they are transferred to a support. This support may be of stiff paraffined paper with holes punched in it, or similarly treated thin cork stoppers provided with holes. The seedlings are placed in the holes, supported by a little cotton.

The procedure of transplanting seedlings to these supports has often seemed to hinder the growth of the plants especially when the operations are performed by elementary students in the laboratory, or even by those with more experience. The delicate seedlings are apparently very easily injured at this stage of growth.

A method which avoids this difficulty has proved very successful in class work at the University of New Hampshire. The main features of the method are shown in figure 1. The container may be an ordinary quart fruit

FIG. 1. Seedling support for nutrient culture work.

[1] New Hampshire Agricultural Experiment Station scientific contribution no. 73.

jar. The support is made from a circular piece of mosquito netting with a diameter about 6 inches greater than the mouth of the jar. This mosquito netting is impregnated with hot paraffin to prevent molding, and is then fastened over the mouth of the jar by means of a rubber band, with the support adjusted to form a pocket about 1.5 inches in diameter, which dips into the jar. The pocket is filled with washed sand of particles large enough not to pass through the netting. The seeds, previously selected for plumpness, are sterilized by soaking in formaldehyde solution, and allowed to remain in running water overnight. They are then planted directly in the sand, near the surface. The base of the pocket is kept immersed in the nutrient solution at least until the seedlings have grown roots well through the sand into the solution. Changing of the solution is easily accomplished by first drawing off the solution in the jar by suction through a narrow-bore glass tube. This may be thrust between the meshes of the netting at one side, first making a place for it by a pencil point. The fresh solution may then be poured directly through the sand. The method might be adapted for very exact studies by using even more inert material than sand particles such as glass beads or quartz.

The plants shown in figure 1 subsequently grew to the heading-out size with very little attention.

UNIVERSITY OF NEW HAMPSHIRE
DURHAM, NEW HAMPSHIRE

NOTES

Columbus Meeting.—The sixteenth annual meeting of the American Society of Plant Physiologists will be held in Columbus, Ohio, during the week of December 25 to 30, 1939. The sessions will be held beginning on Wednesday, December 27, and extending through December 30. This change from the usual Tuesday opening is necessitated by the occurrence of Christmas day on Monday.

The headquarters of the Society will be the Neil House, Columbus, and the annual dinner, scheduled for Thursday evening, December 28, will be held in the Junior Ballroom of the Neil House.

The various sessions and symposia will be held in buildings of the Ohio State University, with possible exception of the joint session with Section G, A. A. A. S. The various meetings of physiologists will be arranged in adjacent buildings, so that transfer from one program to another will be expedited. It is hoped, of course, that the grouping of papers, as at Richmond, may be continued. The wisdom of parallel programs of selected noncompeting papers was fully demonstrated last year.

The central location of the meeting should make possible a very large attendance. Reservations should be made early, for this meeting is likely to set a record in attendance. For hotel and railway rates, consult the notices in *Science*.

Western Section.—The annual meeting of the Western Section of the American Society of Plant Physiologists was held at Stanford University, California, from June 28 to July 1, 1939. Three important symposia were the major features of the meeting: (1) Availability of nutrients in soils to plants; (2) translocation of solutes in plants; and (3) growth. All of these symposia were well attended.

In the symposium on translocation, Dr. K. ESAU discussed the anatomy of vascular tissues, using slides of photomicrographs that were exceptionally good. Dr. C. W. BENNETT discussed the translocation of viruses in plants. Viruses in general move with the food. Dr. D. R. HOAGLAND and Dr. J. P. BENNETT reported on experiments in which they used radioactive elements, which, among other things, very clearly showed that the major salt movement is through the wood, and that considerable lateral movement takes place from wood to bark. The various concepts of the mechanism of translocation of organic materials were discussed in papers by Drs. O. F. CURTIS (read by Dr. J. R. FURR), F. W. WENT, and A. S. CRAFTS. In the extensive discussion that followed, the conclusion was reached that one must distinguish between the four following types of translocation: (1) The "upward movement"

which takes place through the wood, and is mainly caused by transpiration; (2) the "downward movement" which takes place through the sieve tubes, and is caused by pressure flow; (3) movement from cell to cell, in which protoplasmic streaming may aid the diffusion through plasmodesmata; and (4) polar movement of auxin, which possibly takes place along interfaces.

The symposium on growth began with a discussion by Dr. A. S. FOSTER of the morphological aspects of meristematic tissues. Dr. A. R. DAVIS reported on the nutritional factors in growth, in which he especially stressed accumulation and the essential elements. Dr. J. BONNER discussed the rôle of vitamin B_1 in plants, which is now considered to be an important phytohormone. Dr. J. VAN OVERBEEK discussed some of the many aspects of the rôle of auxin in plant growth.

Of the submitted papers, those of Drs. D. R. HOAGLAND, D. I. ARNON, P. R. STOUT, T. C. BROYER, O. BIDDULPH and others on radioactive elements as indicators of accumulation, movement, distribution, etc., were of outstanding interest.

The officers elected for the coming year are: Chairman, Dr. W. M. ATWOOD, Oregon State College; vice-chairman, Dr. A. S. CRAFTS, University of California, Davis; and secretary, Dr. J. VAN OVERBEEK, the California Institute of Technology.

Life Membership Committee.—Following the constitutional regulations governing the CHARLES REID BARNES life membership awards, President J. W. SHIVE has appointed a committee whose duty and privilege it will be to select the sixteenth recipient of this award. The committee is composed of the following members: Dr. R. P. HIBBARD, Michigan State College; Dr. PHILIP R. WHITE, The Rockefeller Institute for Medical Research, Princeton; Dr. GEORGE W. PUCHER, Connecticut Agricultural Experiment Station; Dr. GEORGE P. BURNS, the University of Vermont; and Dr. EARL S. JOHNSTON, the Smithsonian Institution, chairman of the committee. It has been customary for the president to announce the decision of the committee and to make the award at the time of the annual dinner. No one will want to miss being present when the life membership is awarded, for these occasions have become notable events in our Society history. Annual dinner tickets will be available at the registration room of the A. A. A. S.

Chemical Methods Report.—Owing to unforeseen difficulties, it has been necessary to postpone for one quarter, the publication of the report of the chemical methods committee which was planned for October, 1939. The report will be placed in the January, 1940, number of PLANT PHYSIOLOGY.

Errata.—At the close of the table of contents of volume 14, a small

number of errors, reported by authors, have been corrected. Readers are again urged to record these marginally at the places where the errors occur. In this way the errors become harmless. We wish to express our sincere appreciation to the too few authors who have been thoughtful enough to assist us in detecting some of the mistakes. We wish that all authors would proof-read the finished paper, and report, even when they find no errors. The constant press of future requirements prevents the editors from performing this function as they should.

Seventh International Botanical Congress.—The Seventh International Botanical Congress is to be held in Stockholm, Sweden, during the summer of 1940. The dates selected are from about the 17th to the 25th of July. The Secretary of the Congress is Dr. HUGO OSTVALD, Lantbruckshögskolan, Uppsala. A preliminary program of the Congress may be obtained by addressing the Secretary. The permanent program, it is hoped, may be ready for distribution by the first of the year, 1940. Dr. H. LUNDEGÅRDH is Recorder for the Section on Plant Physiology. He may be addressed also at the Lantbruckshögskolan, Uppsala. It is hoped that conditions may remain favorable for a large attendance at the Congress by American delegates.

Back Number.—The editor has received a rare number of PLANT PHYSIOLOGY from a member of the Society, who offers it for sale at $2.50, the usual price for current single numbers. The number is volume 1, no. 3, July, 1926. This volume has been out of print for many years. Here is an opportunity to complete a broken file, if you lack this number.

Lost Numbers.—It has been customary to supply free of charge a copy of any number of PLANT PHYSIOLOGY to members and subscribers in case of nondelivery for any cause. In view of present conditions throughout the world, it is not possible to guarantee delivery. The edition of the journal is limited, of course, by our funds for publication; in case of serious losses of mail addressed to foreign countries, as, for instance, on ships at sea, it would be impossible to replace the lost copies. We will make every effort to deliver the issues to all members and subscribers, but cannot assume responsibility to replace lost copies under present world conditions. This does not apply at present to domestic subscribers and members of the American Society of Plant Physiologists.

Harold Mestre.—In the death of Dr. HAROLD MESTRE on September 9, 1939, at the age of 55 years, the American Society of Plant Physiologists has lost one of its devoted members. Dr. MESTRE was usually present at the

annual meetings, and often took part in animated and thoughtful discussion of the problems that fell in his own field of interest. He will be greatly missed by his colleagues and friends.

Born at Mamaroneck, New York, on August 16, 1884, MESTRE entered collegiate training with much more than the usual maturity. He sought his higher training in California, where, at the age of 39, he received from the University of California, his degree of Bachelor of Arts. He became a teaching fellow and assistant in zoology at the University of California after his graduation, and for several years was assistant and associate professor of biology at the California State Teachers College at Fresno. Entering Stanford University as a fellow in 1927, he pursued his graduate work intensively, and received his Ph.D. degree in 1929. After receiving his degree he was acting assistant professor and assistant professor of biophysics at Stanford for four years. In 1934 he entered Yale University as an honorary fellow in the Medical School. During the last two years he had been at Bard College, Columbia University, at Annandale-on-Hudson. Here he was professor of biophysics, director of studies, and in 1938 became dean of the college.

His main contributions were in the field of radiation, with special reference to spectrophotometry of leaves and algal cultures as related to photosynthesis, photometrical studies of bacterial suspensions, etc. His death is a distinct loss to American botany, particularly to plant physiology, because there are too few accomplished biophysicists in the field of the plant sciences. The memory of his accomplishments will remain as an inspiration to others who may be struggling to advance in the same field.

Advances in the Chemistry of Natural Organic Substances.—The second volume of the *Fortschritte der Chemie organischer Naturstoffe* has been published by Julius Springer, Wien. The general sponsorship of this series of reviews was given in the January, 1939, number of PLANT PHYSIOLOGY. The second volume contains nine reviews of great interest to all biochemical investigators. The first review concerns lignin, which is discussed by K. FREUDENBERG, whose investigations in this field have been numerous and valuable. His short summary is especially interesting to the general student.

Lichen substances are reviewed by Y. ASAHINA. Five groups of compounds are included; the fatty materials, aromatic derivatives of the benzol series, depsides, depsidones, and anthraquinone derivatives.

The flavins are monographed by H. RUDY, most of the attention being given to lactoflavin. A general discussion is followed by synthetic procedures, and a discussion of the co-enzyme function of flavins.

Other reviews include the chemistry of the iodine compounds of the thyroid, by C. R. HARINGTON; the structure and synthesis of vitamin C

(ascorbic acid) and its analogues, by E. L. HIRST; recent methods of oligosaccharide syntheses, by G. ZEMPLÉN; chitin and its decomposition products, by G. TÓTH; tobacco alkaloids, by E. SPÄTH and F. KUFFNER; and spectrochemistry of fluorescence in the study of biological products, by CH. DHÉRÉ. Six of the papers are in German, two in English, and one in French.

These reviews are well prepared, and thoroughly up to date. They are most valuable in giving the reader a general, yet specific, survey of the recent progress in biochemistry of the substances reviewed. The progress of recent years has been so rapid, and so far reaching, that without such integrating discussions one loses the general significance of research in a maze of details. The price of volume 2 of the *Fortschritte* in cardboard binding is RM. 28. Orders should be sent to the publishers, in Vienna.

Growth and Movement.—The second volume of a *Lehrbuch der Pflanzenphysiologie* by E. BÜNNING, K. MOTHES, and F. VON WETTSTEIN, bears the title *Die Physiologie des Wachstums und der Bewegungen.* Dr. E. BÜNNING is the author of this volume. The text falls into three parts, the first of which is a general discussion of rest and activity, the energetics and course of growth, and the mechanisms and catalyzers of extension and plasma building. The second part deals with the physiology of the mechanisms of movement, such as turgor, flagellae, surface energy, plasma streaming, wall tensions, and imbibitional movements. The final section treats of the physiology of stimuli. These include mechanical, radiational, temperature, electrical, gravitational, chemical, and autonomous stimuli and the plant's responses to them. The concluding chapter considers some of the general problems of stimulation and growth response physiology. The discussions are of exceptional interest because of the increasing application of hormone concepts to the explanation of the physiology of growth and movement. The book is profusely illustrated, with 233 text figures. The price of the work bound in paper is RM 18, or RM 19.8 bound in cloth. The publishers, Julius Springer, Linkstrasse 22–24, Berlin W 9, will be pleased to receive orders for this work.

Earth's Green Mantle.—A charming volume by SIDNEY MANGHAM, Professor of Botany in University College, Southampton, bears the title *Earth's Green Mantle.* Works suitable to the layman as well as student are relatively few. MANGHAM tells the story of plant life in eleven chapters, describing the origin and development of plants, the tools by which the secrets of life are discovered, such as microscopy and chemical examination, and then the plant is presented as a machine, whose structures and functions are described under such attractive titles as the machine in the making, the machine in action, mass production and new models, and engine troubles.

The final chapter on progress and prospects presents a generous point of view from which the layman may appreciate the spirit of botanical progress. We recommend this work as one which may be used to create enthusiasm for botany among those who have no technical background for it. A reading list offers additional suggestions for the more complete enjoyment of plant life. The Macmillan Co. offer this unusually interesting work at $3.50 per copy. It is excellent for gift purposes.

Dictionary.—A *German-English Science Dictionary* for students of agricultural, biological, and physical sciences has been published by the McGraw-Hill Book Co. The author is LOUIS DE VRIES, Professor of Modern Languages at Iowa State College. This handy volume will be welcomed by many a student who is struggling for mastery of German scientific literature. The only criticism which one can offer to such works is that they are seldom complete enough for all of the requirements; words omitted, or definitions not versatile enough. There are about 48,000 words included, however, which is a very good start. Students who find any inadequacy should accept the author's invitation to submit lists of terms which ought to be included. Just at a chance, the editor looked up Photosynthese as a key word in botany; but although there are a number of Photo-compound words listed, this one was not. Perhaps it was thought to be too easy to need listing. It isn't much easier, however, than Photo-graphie which is listed. A good test will be to use the dictionary on a serious chapter in some recent German text on Pflanzen-physiologie; but keep a pad at hand to list the terms to be added. A later edition can remedy any serious sins of omission. The author has made a fine start at a good, usable dictionary. The quoted price is $3.00 per copy.

GENERAL INDEX[1]

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